Electron Microscopy & Photography

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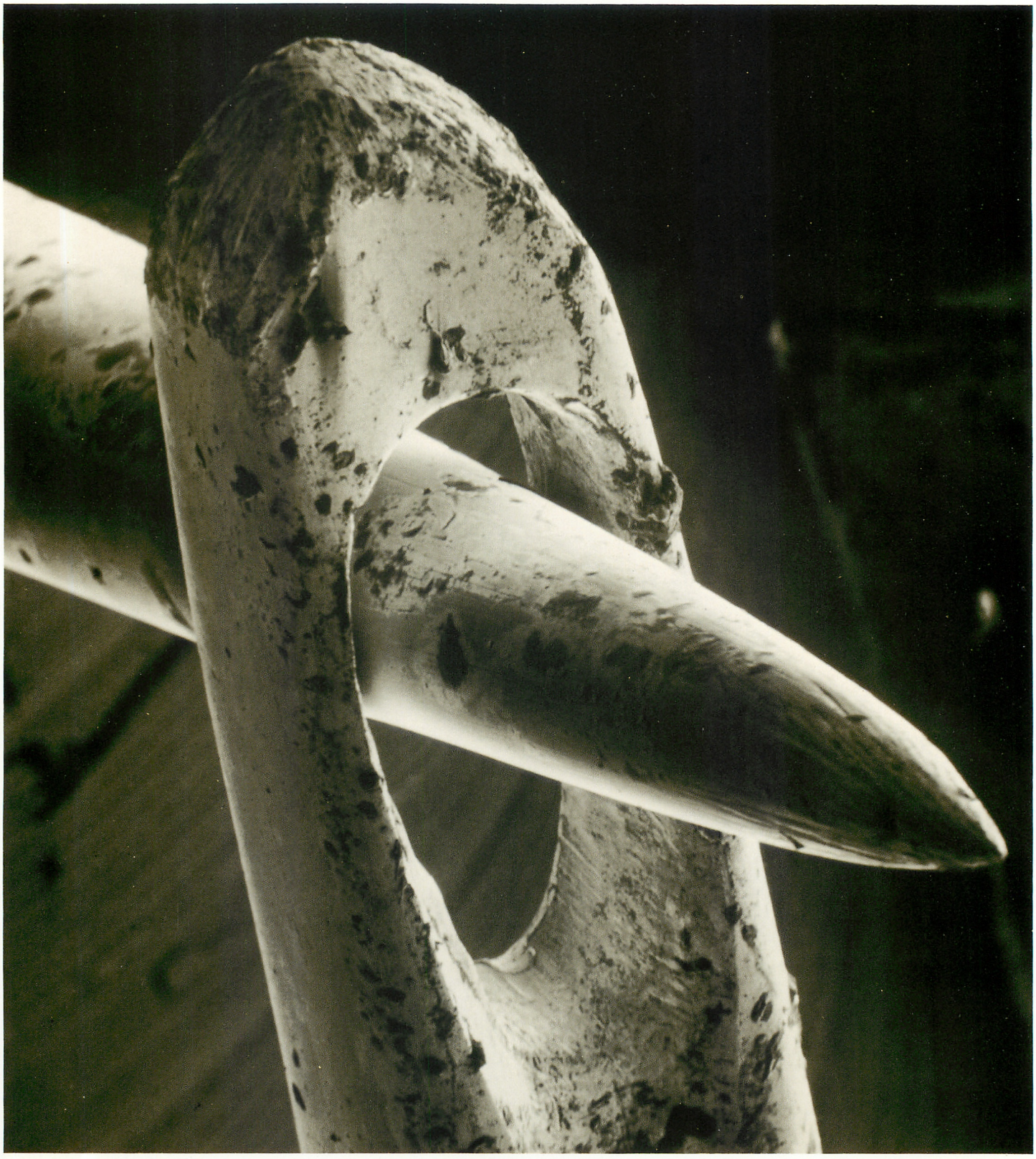
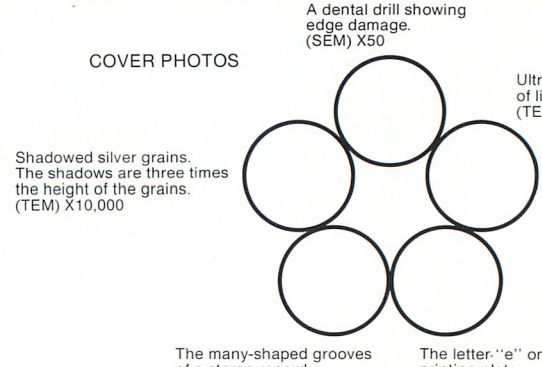


Figure 1—Scanning electron micrograph—the point of a needle through the "eye" of a needle (150X).

All of the scanning electron micrographs in this publication, except for Figure 19, were supplied by the Industrial Laboratory, Eastman Kodak Company, Rochester, New York 14650.

COVER PHOTOS



Electron Microscopy and Photography

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About This Publication

Eastman Kodak Company has been aware of the needs of the electron microscopist from the early years of the electron microscope to the present day. In fact, when electron microscopes in the United States numbered only three, our research laboratory was the proud owner of one of these. Now, however, electron microscopes are found throughout the world. They have become important in revealing detail beyond the resolution limit of the optical microscope. They have found wide applications in research, industry, and medicine, and the list of applications increases with each new installation. This publication covers many aspects of electron micrography, and emphasizes the photographic materials and methods to be used.

Included is a general description of the two types of electron microscopes—the transmission electron microscope (TEM) and the scanning electron microscope (SEM). These instruments are simply explained (although they are not simple) with diagrams and illustrations. The basic principles of the electron gun and magnetic lenses are illustrated. These descriptions, in conjunction with the books listed on the reference page, will give the new or experienced microscopist a better understanding of his instrument and its accessories.

Because sample preparation is an ever-changing area where experimentation and knowledge of new methods will prove extremely helpful, we recommend that you consult the books listed on the reference pages and also attend electron microscopy symposia to keep up to date. If you uncover a successful method, have it published in order to aid your colleagues.

The main section of this book deals with photography in relation to electron microscopy. First, photographic theory is explained (emulsion sensitivity to electron exposure, the meaning of DQE, graininess patterns, etc). Second, the practical methods of photography, such as processing, enlarging, printing, and various special methods to help you get the best print from your negative are covered. In this section we also list Kodak films, plates, and papers for the microscopist. To simplify the comparison and selection of film and plate materials, all are listed on a chart in the center of the book. This chart includes information about each of the products.

The final section of this publication includes a list of electron microscopy suppliers, current reference books for both the transmission and scanning electron microscopes, and Kodak publications that should be of interest to the microscopist.

Using the Metric System

In all scientific fields, the use of the metric system has been universal. To standardize the general usage of all measures, the metric system has been included within a larger body of measures known as the International System of Units (abbreviated SI from the French *Systeme International d'Unites*), adopted in 1960. The SI includes units of time, force, work, power, electric current, and others, besides the original metric unit. These units have been adopted by the National Bureau of Standards (NBS), the National Aeronautics and Space Administration (NASA), and by the American Society for Testing and Materials (ASTM).

Because of the new standards, several changes have been made that affect the optical and electron microscopist. The word "micron," with its Greek symbol μ (equal to one millionth of a meter), has been renamed micrometer (designated as μm), and the millimicron (mμ) has been replaced by nanometer (nm). One change that will affect the electron microscopist is the elimination of the angstrom unit (1A = 0.1 nm or 10,000A = 1 μm), a term that has been used for years to describe interatomic distances and resolution of electron microscopes. The angstrom was eliminated because it did not conform to metric classification. However, we recognize that most microscopists still use the angstrom unit so, in this publication, we will use nanometers as the main measurement with the corresponding angstrom unit in parentheses. Table 1 lists the units of length that will be used throughout this book.

Table 1 **COMPARISON OF UNITS OF LENGTH**

	EQUIVALENT UNITS OF LENGTH						
Unit	**Meters (m)**	**Centimeters (cm)**	**Millimeters (mm)**	**Micrometers (μm)**	**Nanometers (nm)**	**Angstrom (A)**	**Inches (in.)**
1 meter (m)	1	100	1000	1,000,000	1,000,000,000	1×10^{10}	39.37
1 centimeter (cm)	0.01	1	10	10,000	10,000,000	1×10^{8}	0.3937
1 millimeter (mm)	0.001	0.1	1	1000	1,000,000	1×10^{7}	0.03937
1 micrometer (μm)	0.000001	0.0001	0.001	1	1000	10,000	0.00003937
1 nanometer (nm)	0.000000001	0.0000001	0.000001	.001	1	10	0.00000003937
1 angstrom (A)	1×10^{-10}	1×10^{-8}	1×10^{-7}	.0001	0.1	1	0.000000003937
1 inch (in.)	.0254	2.54	25.4	25,400	25,400,000	2.54×10^{8}	1

Note: To use this table, the unit on the far left equals all units in that row. For example, 1 centimeter (cm) equals 10 millimeters, 10 million nanometers, or 0.3937 inches.

Introduction

Man is forever probing and searching to be able to see "just a little more," to resolve finer detail, to separate just a few more lines per millimeter, and to see things he has never seen before. These were some of the reasons for Anton van Leeuwenhoek's invention of the optical microscope in 1675. The microscope was the scientific toy of that century and was called a "flea" or "fly" glass because it was used primarily to observe insects. Leeuwenhoek, with his microscope of double convex lenses (which he ground by hand with diamond dust), was able to demonstrate the circulation of the blood, to discover bacteria and protozoa, and to attain magnification of 160 diameters. What great excitement this must have brought forth in the medical world of that day!

The optical microscope has been improved through the years by many great men continuing this same search for new knowledge. Improvements in lenses, microscope design, and illumination have now achieved the top magnification and resolution limit of the optical microscope. Similarly, the early studies (1932 through 1934 by Ruska and Knoll) of the electron and its characteristics were climaxed by the observation of an image upon a fluorescent screen. This image represented a mere shadow of the specimen. With advances in Germany (Siemens and Halske) and in Canada (Toronto University*), the transmission electron microscope was developed for research work, and a standard commercial instrument became available in the 1940's.

In the preface to *The Electron Microscope* (1942), authors Burton and Kohl compared the early research in electron microscopy to the travels of Gulliver, in *Gulliver's Travels, Voyage to Laputa*. In his travels, Gulliver met a projector (researcher) who had been *working eight years on a project extracting sunbeams out of cucumbers*. These sunbeams were then sealed in vials and used to warm the raw air in colder weather. The parallel with the projector of Laputa and the electron microscope investigator of Toronto in 1941 is that the latter had been *working eight years on a project of taking magnified pictures without light*. This appeared to be just as foolish to a present-day Gulliver as the sunbeams were to the original. As we know, however, one success led to another and a whole new world of research has opened up.

Figure 2—The first electron microscope built in North America. It was constructed at the University of Toronto and is now on display at the Ontario Science Centre, Toronto, Canada. (Illustration courtesy of the Ontario Science Centre.)

Why was there a need for an electron microscope? Let's investigate what has been accomplished in the field of electron microscopy and see why the transmission electron microscope (TEM) has developed so rapidly. Let's also consider the scanning electron microscope (SEM), whose growth has increased even more rapidly. We will discuss both types of electron microscopes, why they were necessary, and how they differ from one another.

THE OPTICAL MICROSCOPE

Resolving Power of the Optical Microscope

The eye will separate two objects if they are not closer than 0.1 mm (0.004 inch)

*The original electron microscope constructed in North America (see Figure 2) is displayed beside a modern electron microscopy laboratory at the Ontario Science Center, Toronto, Canada.

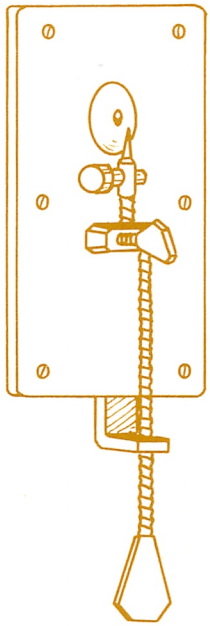


Figure 3—The Leeuwenhoek microscope was operated by placing the object to be viewed on the sharp point. The observer's eye was placed close to the lens, with the sun acting as the light source.

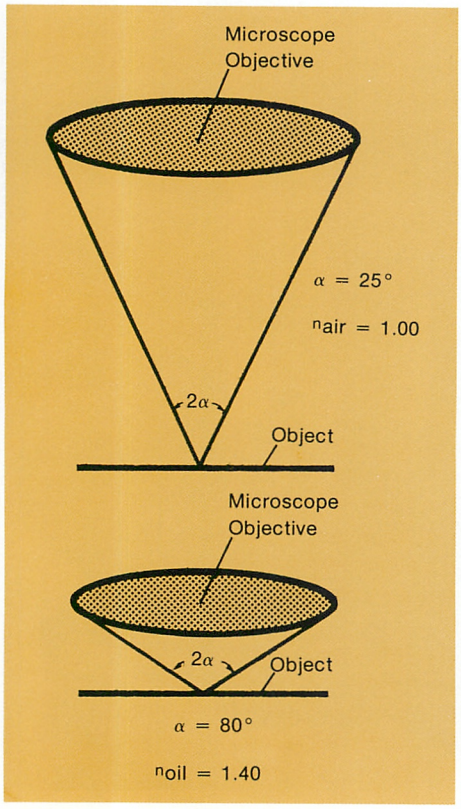


Figure 4—Angular aperture of different microscope objectives.

at a normal viewing distance of 25 cm (10 inches). This is the limiting factor of the human eye and any finer detail than this must be enlarged to be resolved. A simple hand magnifier or a complex compound microscope can be used to enlarge the image. The microscope objective lens possesses the ability to make fine structural detail in the specimen distinct. This ability is expressed in terms of *numerical aperture*, or "N.A." as it is usually called. The numerical aperture is the product of n sine α, where n is the refractive index for the medium through which the light passes; examples, (n)air = 1.00, (n)water = 1.33, and the sine α of one-half the angular aperture of a lens. The sine α relationship which is the ratio of two sides of a triangle is a simple means of expressing the size of an angle as a number. Microscope objectives and condensers are largely designated by this N.A. value. The effect of α is shown in Figure 4. The limits of the objective lens are such that α cannot be greater than 90 degrees, and that the object space, even if filled with an oil of greater refractive index than air, can only reach an N.A. of 1.4. The resolving power of the light microscope is also limited by the wavelength of the illuminant. To explain this more fully, the resolving power (the smallest distance between two points which can be seen as separate on the image) of the optical system is given by the formula $R = \frac{\lambda}{2\ N.A.}$ where R is the distance between distinguishable points (in nanometers), λ is the wavelength of illuminant (in nm), and N.A. is the numerical aperture imprinted on the microscope objective.

The finest optical resolving power is obtained with ultraviolet radiation, which represents the shortest usable wavelength in optical microscopy. In the visible region of the spectrum, blue light has the next shortest wavelength, then green, and finally red. If white light is used, the applicable wavelength is that for green—which is the middle of the visible spectrum and the region of highest visual sharpness.

The resolving power for some common apochromatic objectives can be found with the formula $R = \frac{\lambda}{2\ N.A.}$. The following table lists the resolving powers. The illumination for these examples was green light (dominant wavelength 550 nm).

Resolving power for the highest N.A. (1.40) microscope objective with light of different wavelength is shown in this table. Resolution improves with shorter wavelengths of light. It is possible, for example, to resolve particles as small as 130 nanometers (1300 A) by using ultraviolet radiation in the optical microscope.

Other Limiting Factors (Optical System)

The depth of field and the limits of magnification are several factors that are very restrictive in optical microscopy. The depth of field in microscopy, as well as normal photography, is the distance from the nearest part of the *subject* to the farthest part of the subject that is in acceptable focus. Do not confuse this terminology with "depth of focus"—which is the distance the *image* can be moved and still be in focus. In photomicrography, the higher the magnification and the higher the N.A., the shorter the depth of field. As the N.A. is increased, the aperture of the objective becomes wider and depth of field is reduced. This is similar to a photographer "opening up" his lens, which reduces the range of distance in which the subject is in focus. For example, an objective with an N.A. of 0.25 will give a depth of only 100 nm (0.1 micrometer). Therefore, why not use a low N.A. objective to get more depth? The answer is that R depends upon N.A. and the usable magnification of any objective is equal to approximately 1000 times its N.A. Therefore, the usable magnification of N.A. 0.25 and 0.95 objectives are approximately 250× and 950×. Any magnification higher than this will not resolve more detail and will give only "empty magnification." Therefore, if higher magnification is needed, then a higher N.A. must be used, although this causes a decrease in depth of field.

Resolving Power for Apochromats

Magnifying Power	10×	20×	47.5×	90× oil
Numerical Aperture	0.30	0.60	0.95	1.40*
Resolution (nm)	920	460	290	190

*Highest N.A. for an apochromatic objective.

Change in Resolution with Wavelength (Optical Microscopy)

	Green	Blue	Ultraviolet
Wavelength (nm)	546	436	365
Resolution (nm)	190	160	130

The Electron Microscope

INTRODUCTION OF THE ELECTRON

Due to the detail-resolving limitation of the illuminant in microscopy, new sources of illumination have been sought for many years. Experiments with x-rays of extremely short wavelengths (0.5 nm) were made, but because these rays could not be focused, they were of little practical use. However, in 1923 and 1926, developments were made upon which the modern electron microscope is based. Louis de Broglie advanced his theory of the dual nature of the electron. He determined that the electron, which is a minute particle of matter charged with the smallest known quantity of negative electricity, had the characteristics of both a particle and a wave (similar to light). In addition, Hans Busch, of the University of Jena (Germany), demonstrated in 1923 that a beam of electrons traversing a magnetic or electric field will converge to a focus, therefore proving that electron lenses were possible. In 1932, M. Knoll and E. Ruska (Germany) developed the first magnetic electron microscope. Utilizing a 60,000-volt beam and short-focal-length magnetic lenses, they produced images on a fluorescent screen. Advances in Germany were very rapid after this breakthrough and subsequently spread, first to Canada and then to the United States. Let's examine the impact this development had on detail resolution in the instrument.

As electrons are accelerated through a potential difference of V volts, they have a wavelength (λ) equal to $\frac{1.23}{\sqrt{V}}$ nm. Therefore, a 50 kV electron has a λ of 0.0055 nm. The aperture of the magnetic lenses as compared to the microscope objective is extremely narrow ($< \alpha = 1°$) because of the inherent spherical aberrations. However, if we enter these figures in the resolution formula $R = \frac{\lambda}{2 \text{ N.A.}}$, then Resolution $= \frac{0.0055 \text{ nm}}{2(.01)} =$ 0.27 nm. This resolution is three orders of magnitude (10^3) greater than the white-light illuminant in the optical microscope. In fact, resolving powers of 0.3 nm (3 A) are guaranteed on many electron microscopes. Other instruments have demonstrated under optimum conditions the ability to resolve detail that are separated by 0.1 nm (1 A).

Figure 5—A transmission electron micrograph of a film surface, (7500×) prepared by the preshadowed replica method. The large, matte particle's height can be measured by the length of its shadow.

The focal length of the objective lens in a 100 kV electron microscope can be about 1.1 mm. In photographic terms, this lens approximates a stopped-down lens. This factor greatly enhances the depth of field, as illustrated by the extreme sharpness of detail in electron micrographs of rough surfaces (see Figure 5). This depth of field is several hundred times greater than that with a high-N.A. optical microscope.

With the resolving power of the optical microscope at 100 to 200 nm (1000 to 2000 A) under the best optical conditions, the optical microscope is limited to defining only the smallest bacteria. However, with the electron microscope, the resolving power has been increased to 0.1 nm (1 A), thus small molecules and even individual atoms can be resolved. This instrument can examine particles, surfaces, and tissues with finer resolution and clarity than had ever seemed possible. Most modern electron microscopes use the general principles of the electromagnetic lens (with improvements) and other features that will be explained in the following section.

THE TRANSMISSION ELECTRON MICROSCOPE (TEM)

The simplest electron microscope is very similar to the light microscope (see Figure 6). Both have condenser lenses to concentrate the incident beam upon the specimen; the beam passes through the specimen and is focused by the objective and projector lens to form an enlarged image on a fluorescent screen. However, the electron microscope is much more complex than the light microscope. For the electrons to reach their acceleration speed, they must operate in a high vacuum (10^{-3} to 10^{-6} torr, 1 torr = 1 mm of mercury at 0 C). The whole electron microscope system, in-

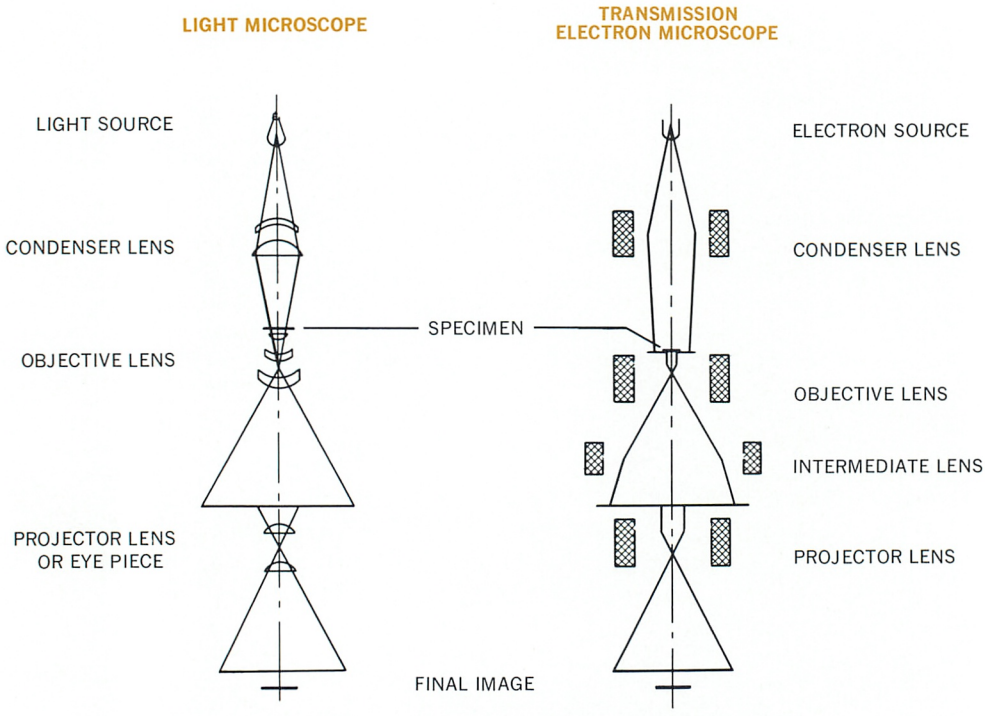


Figure 6—Simplified drawing illustrating the similarity between the light microscope and the transmission electron microscope.

cluding the fluorescent screen and photographic material, is a hollow tube evacuated by means of mechanical and oil-diffusion pumps to a pressure of 10^{-6} torr or lower. The electric power supply unit that generates the accelerating voltage provides the objective lens and the accelerating voltage with an extremely stable source. This power supply, with its solid-state components, is usually placed some distance away from the instrument to prevent any stray magnetic fields from interfering with the magnetic lens systems. Most instruments are equipped with safety devices that will not allow the high filament voltage to be switched on until the proper vacuum is maintained.

The magnetic lenses of the electron microscope consist of a circular magnet with an annular pole piece separated by a narrow gap (see Figure 8). The strength of the magnetic field across this gap determines the focusing of the electron beam. The magnet is either permanently magnetized or is an electromagnet to a variable current source. Changing the amount of current to the lens gives the effect of a continuous, variable-focal-length lens. In fact, the instrument approaches a zoom-lens system. Accelerating voltages can be adjusted

Figure 7—The Hitachi Transmission Electron Microscope HU-12 (courtesy of Perkin-Elmer Corporation).

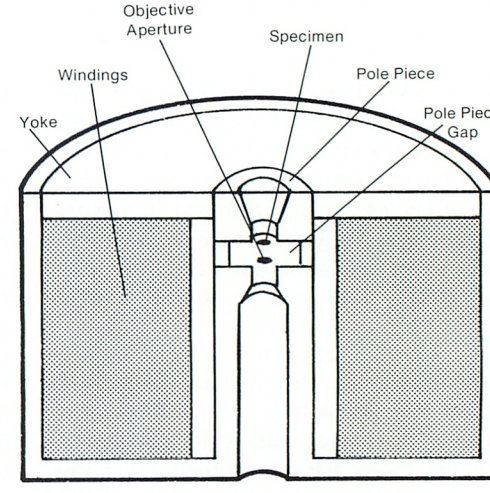


Figure 8—A cross section of the magnetic objective lens. The yoke and winding produce the magnetic field in the pole piece gap. The objective aperture and location of the specimen are marked.

on most instruments from about 40 to 100 kV. Lower accelerating voltages are used for examining extremely thin specimens and producing increased specimen contrast. However, due to internal heating of the specimen, some specimen damage may be evident. High accelerating voltages are used for penetrating thicker specimens. A cross section of a transmission electron microscope is shown in Figure 9.

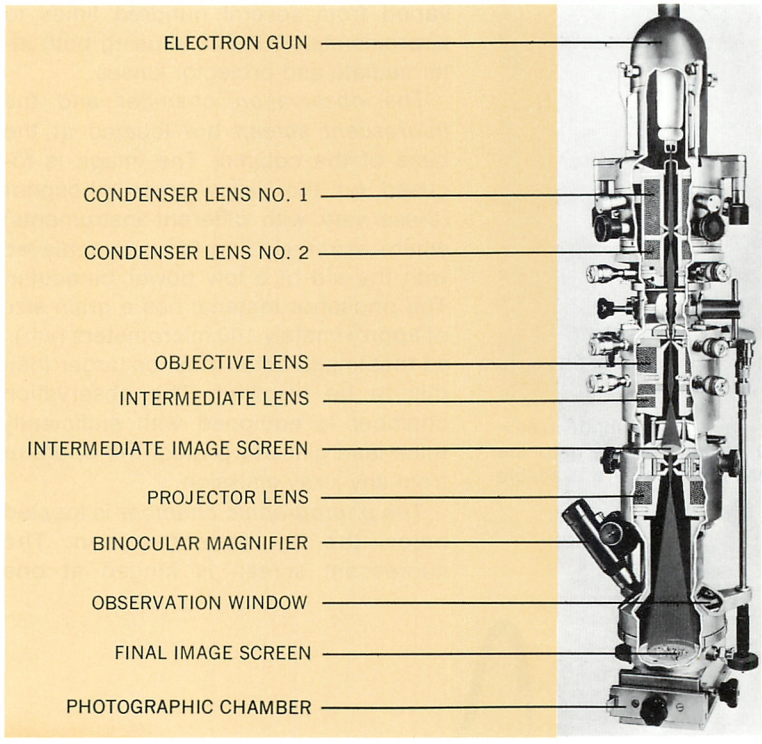


Figure 9—A cross section of the transmission electron microscope (courtesy of Siemans Corporation).

Components of the Transmission Electron Microscope (TEM)

The electron gun consists of a porcelain insulator, at one end of which is clamped a filament of tungsten wire bent into a V or hairpin shape and surrounded by a shield (called the Wehnelt cap). This shield has a circular aperture 1 to 3 mm in diameter, centered at the filament apex. Proper alignment of this filament is important. Total beam intensity from cathode to anode may be from 10 to 400 microamperes, but only a small fraction of this gets to the specimen.

Recently, a normal hairpin-shaped tungsten filament with a finely sharpened piece of tungsten wire welded to the tip was introduced. These "pointed" filaments, as they are called, produce a higher contrast and better image formation.

The double-condenser lens is capable of a wide range of intensities with a given gun adjustment, and makes possible the reduction of the area illuminated at the specimen. Only the area being examined is illuminated. The two lenses together produce a reduced image of the source, and their strengths can be adjusted to give the proper intensity of illumination. These lenses are aligned to produce an even illumination of the specimen.

The specimen stage (not illustrated) is located just in front of the objective lens; the specimen is inserted into the electron microscope column through an air seal. All specimens have to be placed on a metal grid 2.3 or 3.0 mm in diameter and 0.2 mm thick. These grids are available in copper, nickel, gold, platinum, and woven stainless steel.

The objective lens is the most important lens in the electron microscope. The focal length of this lens may be between 1 to 5 mm with 1.1 mm being a common focal length. The shorter focal lengths result in less spherical aberration and better resolution. Because the imaging beam has its largest angular aperture in the first stage (objective) of the microscope, this lens is the main factor limiting the quality of the recorded image. Any residual astigmatism present in the objective lens is corrected by the use of a *stigmator,* a device that compensates by adding metal to offset the inherent inhomogenity of the lens. This device is a necessity if high-resolution work is to be accomplished. Another important component that is located near the objective lens is the *objective aperture,* which limits unwanted scattered electrons that could degrade the image. This aperture enhances the contrast and is available in several different sizes, with the 20 and 40 micrometers (μm) sizes being the most popular.

The intermediate lens can either magnify or demagnify the image. This may be accomplished by raising or lowering the current to this lens.

The projector lens corresponds to the ocular of an optical microscope. Its function is to form a *real* image on the fluorescent screen and to offer a wide choice of magnifications through a current change. The magnification can be

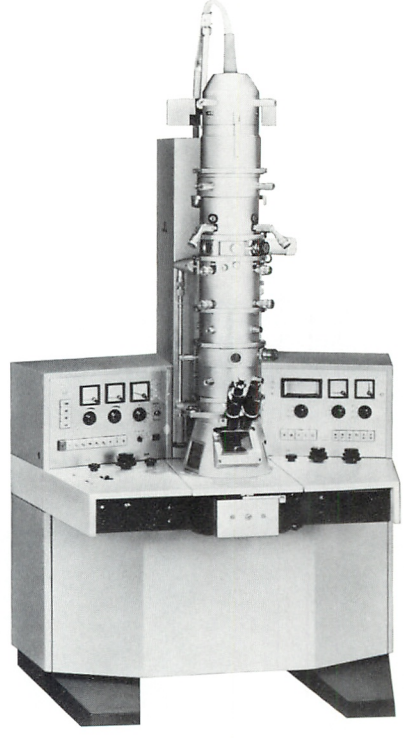


Figure 10—Elmiskop 101 Transmission Electron Microscope (courtesy of Siemans Corporation).

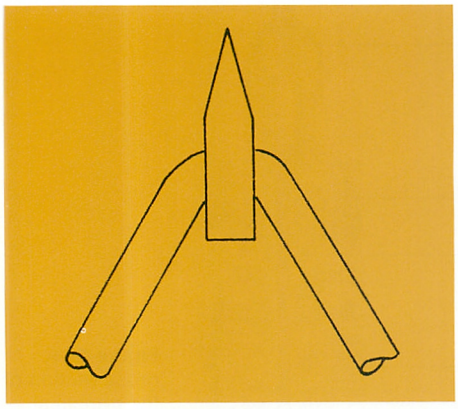


Figure 11—Pointed filaments are made in several ways. A tip is usually welded onto the regular tungsten filament. They are available from EM suppliers.

varied from several hundred times to approximately 300,000× using both intermediate and projector lenses.

The observation chamber and the *fluorescent screen* are located at the base of the column. The image is focused on the yellow-green phosphor (these vary with different instruments) where extremely fine focus is achieved with the aid of a low power binocular. The phosphor material has a grain size of approximately 100 micrometers (μm), so that image detail must be larger than this to be resolved. The observation chamber is equipped with sufficiently thick lead glass to protect the operator from any x-ray emission.

The photographic chamber is located below the fluorescent screen. The fluorescent screen is hinged at one edge, and when it is tilted out of the electron beam, the electron image falls upon the photographic plate or film. There are a variety of different-size glass plates, sheet films, and 35mm and 70mm roll films that are commonly used. These products are listed in the chart on page 24. The positioning of these photographic products is not critical because of the electron microscope's great depth of focus which extends several centimeters above or below the fluorescent screen. The main consideration, however, is the difference in magnification at the different positions. Other accessories (TV-display, automatic image analysis, and image intensifiers) are placed at this end of the column with their sensor device located near or below the fluorescent screen.

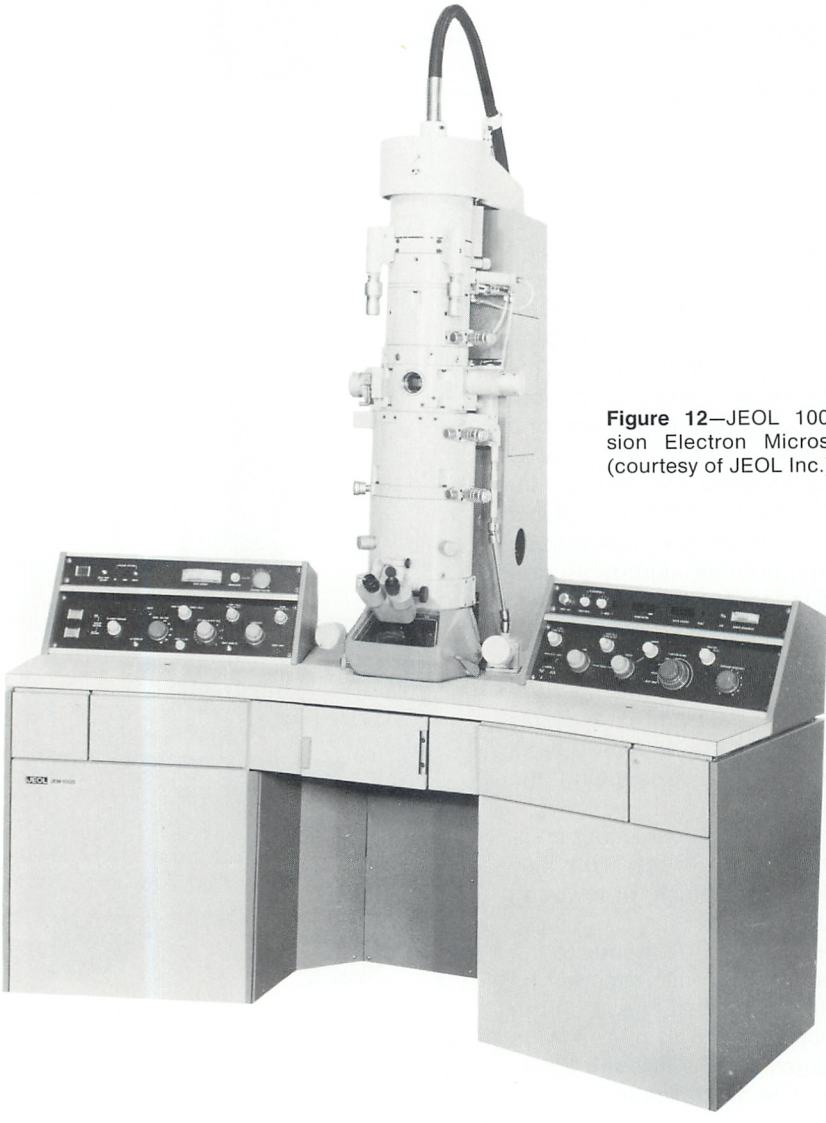


Figure 12—JEOL 100B Transmission Electron Microscope (TEM) (courtesy of JEOL Inc.).

Other Transmission Electron Microscopes

The previous description is of a general type of transmission electron microscope in use today. In addition to this basic unit, there are many modifications of this model that we will only mention. Due to the many improvements and advances each year, the new instruments are becoming smaller (solid-state electronics), more sophisticated (can be utilized for scanning, x-ray diffraction, etc), and more automated (have push-button vacuum systems, TV scanners, and versatile photographic facilities).

High-voltage microscopes (500 kV to 3MeV) have been recently introduced in several countries (England, France, Japan, and the United States). These instruments (resolution: 1 nm (10 A) at all magnifications) are capable of examining and photographing specimens that are approximately 1.0 micrometer or more thick, while normal 100-kV instruments can only penetrate specimens that are in the range of 0.05 to 0.10 micrometer thick. This is a big advantage, especially for applications where ultrasectioning may effect the general structure detail of the specimen.

Another advantage of this instrument is the reduction of beam damage to the specimen due to the great acceleration speed of the electrons. The electrons pass through the specimen without causing internal heat that deforms and contaminates the specimen. This feature has led to the examination of living bac-

teria with this instrument. The bacteria were then removed and placed in a culture dish where they continued to live and reproduce (the radiation had no effect). This may have far-reaching effects on future studies of the life sciences.

Several disadvantages of the high-voltage electron microscope are excessive weight (~ 20 tons), especially the lenses; the massive size of the high-voltage generator; and the x-ray radiation given off (for example, the lead glass in the observation chamber of a 1MeV instrument is 700 mm, or 28 inches, thick). Therefore, protection of the operator becomes a major problem. However, it seems likely that these disadvantages will be overcome as more instruments are built and present instruments are modified.

Some manufacturers market *low-power electron microscopes* designed primarily for quality control testing, for teaching and training facilities, and for any other application where the requirements are not satisfied by the optical microscope. These instruments may have prealigned lenses and relatively few adjustments. Although these instruments are limited in magnification, the resolving power is usually sufficient for many applications. They are well suited for small facilities because of their compact size and ease of operation.

Another electron transmission microscope that is similar to the microscope described above is the microscope that contains *electrostatic lenses.* These lenses differ from electromagnetic lenses (see page 6) in the following aspects; (1) the electron trajectories depend upon a constant ratio between the accelerating voltage and lens voltage; (2) the electrode surfaces are equipotential; and (3) the image does not rotate as the magnification is increased. These lenses are prealigned for ease of operation.

The transmission electron microscope has many applications. Here are a few: cell and tissue examination, bacteria studies, particle size studies, product evaluation, surface studies, smoke and air pollution studies, and research and development projects. There are so many applications that it is impossible to make a complete list, because each facility has its own methods and uses.

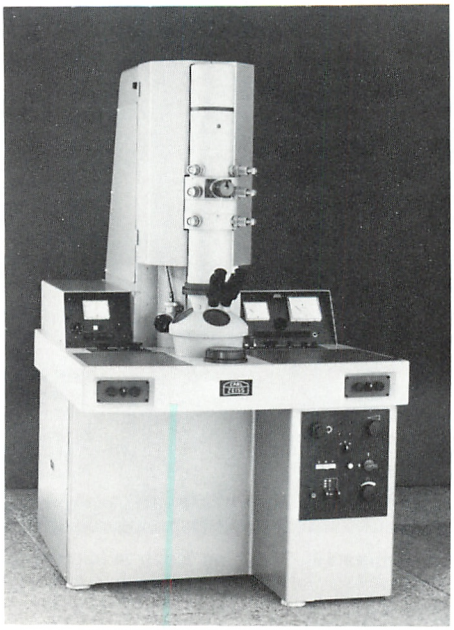


Figure 13—Zeiss EM 9S-2 Transmission Electron Microscope (courtesy of Carl Zeiss Inc.).

However, one factor is common to all of the applications. All specimens viewed in the TEM must be extremely thin (in the range of 100 to 1000 A) or the electron beam cannot pass through the specimen. A thick specimen will usually produce a poor micrograph showing very little detail. The use of replicating or ultra-thin sectioning techniques are the most common methods of examining a thick specimen. A conventional scanning electron microscope can be used to study the surfaces of thick specimens, but its resolution is somewhat limited. The scanning electron microscope views solid or opaque specimens that only need to be coated with an electron-conducting metal to be viewed. The need for this type of instrument, as well as its rapid popularity, will be discussed in the next section.

THE SCANNING ELECTRON MICROSCOPE (SEM)

As with the optical microscope of the 17th century, the scanning electron microscope—SEM—until only a short time ago was the "flea" or "fly" glass of this age. Many thought of the SEM as a novelty instrument with limited application, and oddly enough, the earlier pictures were of flies' eyes, wings, etc. However, times have changed; the instruments have been greatly improved, the applications are becoming more numerous daily, and the illustrations made by these instruments are more exciting than any illustration of the past.

The principles of the scanning electron microscope are as old as the transmission electron microscope. The early

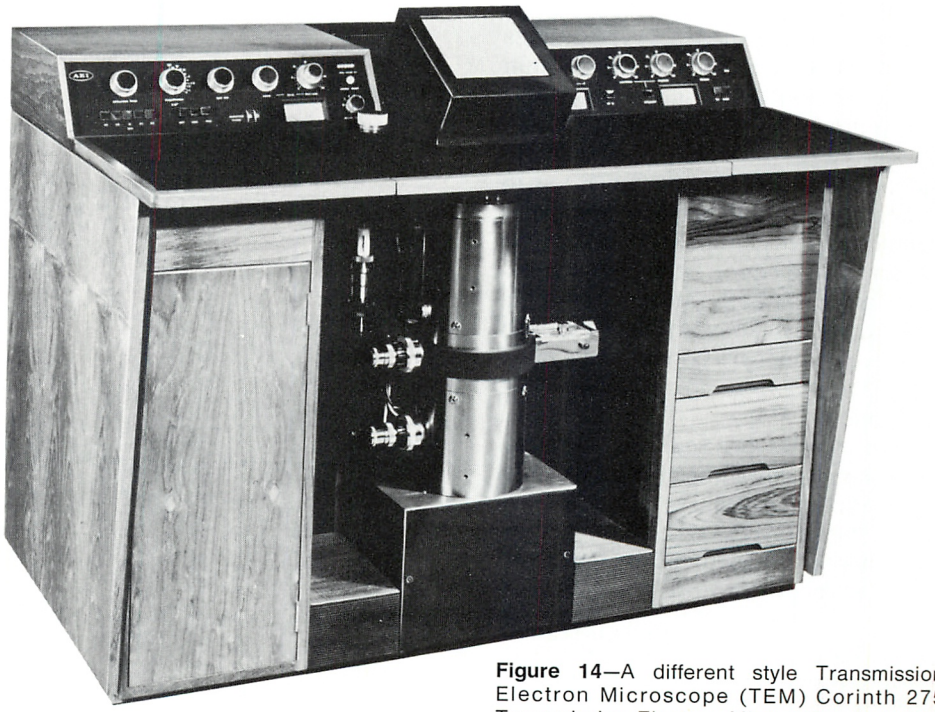


Figure 14—A different style Transmission Electron Microscope (TEM) Corinth 275 Transmission Electron Microscope (courtesy of AEI Scientific).

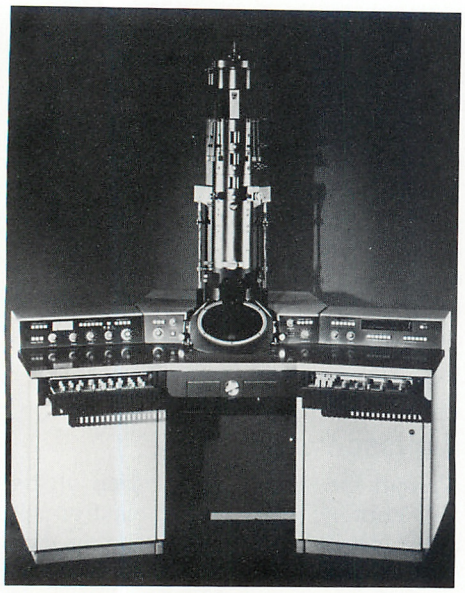


Figure 15—Philips 301 Transmission Electron Microscope (TEM) (courtesy of Philips).

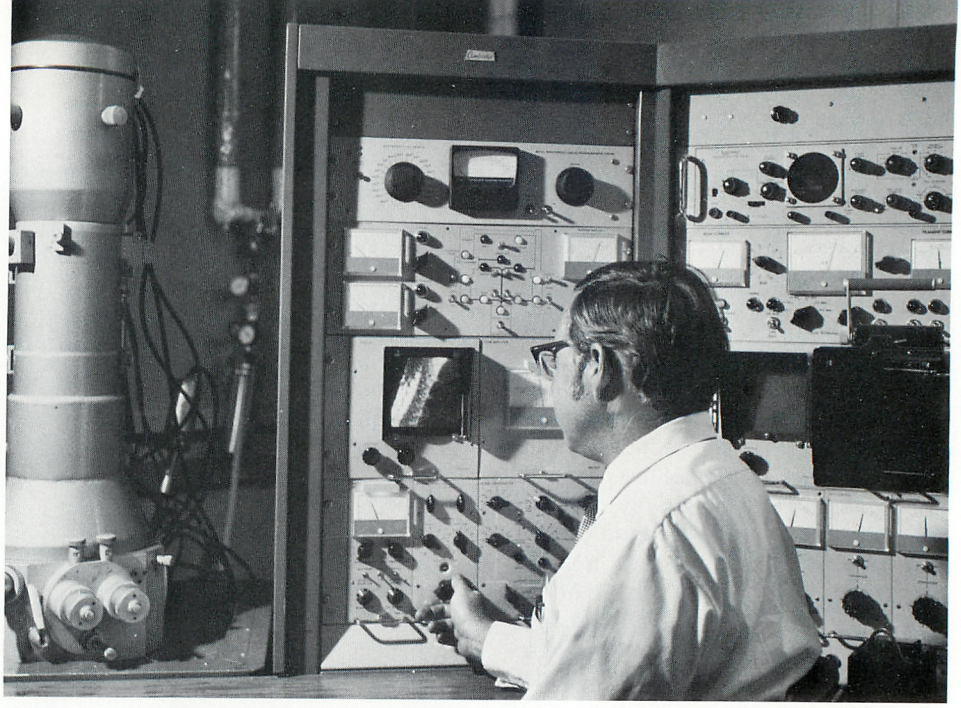


Figure 16—Scanning Electron Microscope (Kent Cambridge Stereoscan) and operator.

experiments, however, encountered many difficulties. The first instruments were developed in Germany in the 1930's, in the United States in the 1940's, and finally in England in the 1950's. It was this final work that led to the almost foolproof commercial instrument that was introduced to the world in 1964 by the Cambridge Scientific Instrument Company. This major development was the work of many (Oatly, Nixon, Pease, etc) at the Cambridge University Engineering Laboratories. Developments have continued since then with other major electron microscope manufacturers introducing their models (see listing on page 47). The availability of instruments in recent years has led to a great number of applications. New techniques, which have no parallel in transmission electron or optical microscopy, are constantly being developed. This is a good indication that the growth and development will continue at a rapid pace. It is an exciting field with wide applications.

What makes the scanning electron microscope different from the transmission microscope? Here is a simple schematic (Figure 17) which shows the fundamental components of the scanning electron microscope.*

*This information is from "Introduction to Scanning Electron Microscopy," W. C. Nixon, *Scanning Electron Microscopy,* 1969, proceedings of 2nd Annual Scanning Electron Microscopy Symposium sponsored by IIT Research Institute.

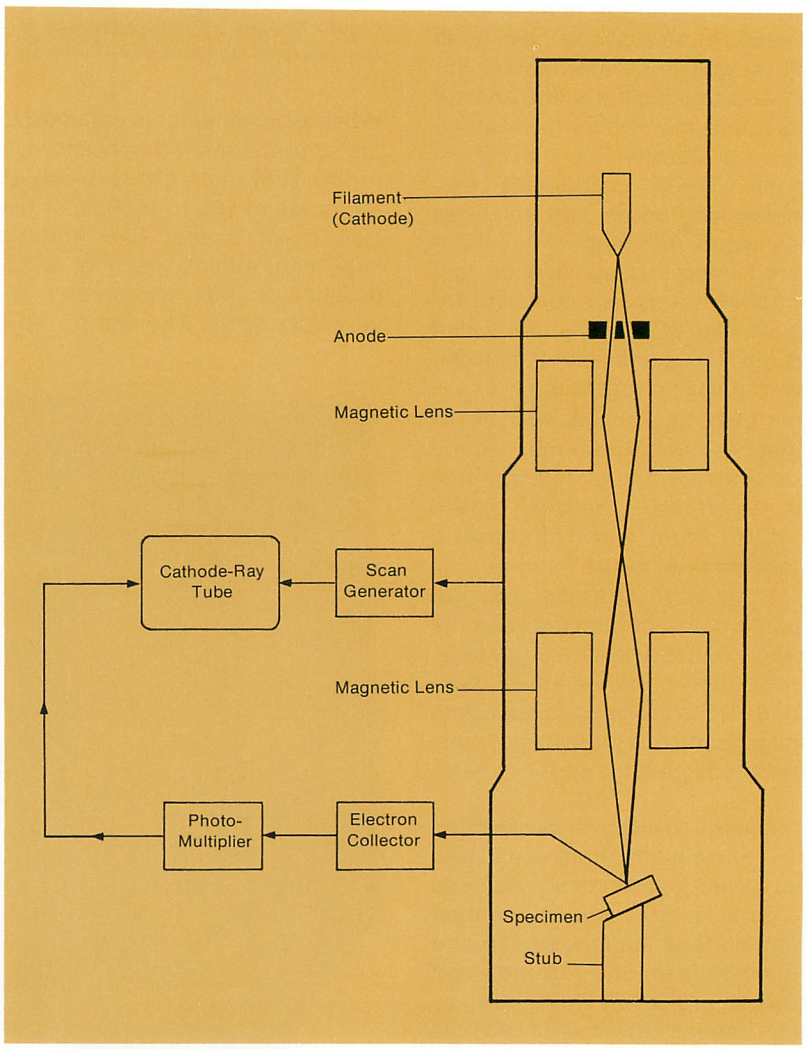


Figure 17—Components of the Scanning Electron Microscope (SEM).

The *electron gun* forms a source of electrons, which are accelerated toward the *anode.* The anode is held at ground relative to the kilovolts on the gun cathode and control grid. These electrons pass through one or more electromagnetic lenses, and the image of the electron source is found in the plane of the specimen after successive reductions. At some points along the path, the electron beam is deflected (1, 2, 3, . . . n times) by fields controlled by the *scan generator.* As a result, the beam is moved over the surface of the specimen in a predetermined form such as a square television-type raster or some other control. These primary electrons produce secondary electrons at the specimen surface and are detected by an *electron collector.* Other events lead to the emission of cathodoluminescence, x-rays, and conductivity effects within the specimen. The collector may be adjusted to deal with any or all of these signals.

The *specimen*, which is mounted on a metallic stub, may be moved in the X, Y, or Z direction, and can be rotated, tilted, heated, cooled, stretched, strained, and experimented on within the instrument. This specimen stage must be free of vibration and all movements must be repeatable.

The *photomultiplier* enhances the collected signal and may also alter or otherwise process the information coming from the specimen as the electron beam is scanned over this surface. The *scan generator* is linked to the display cathode-ray tube (CRT), so that the electron beam in this tube is being scanned in the same way as the electron beam in the main column. However, the scanning power supplied at the main column may be attenuated at any time, while the display tube is scanned over a constant area. As a result, any reduction of the scanned area on the specimen leads to an increase in the viewed magnification which is determined by the ratio of the tube screen face (constant) to the scanned area of the specimen (variable).

The column and the specimen chamber are evacuated to a high vacuum by an independent system of mechanical and oil diffusion pumps. Whenever the specimen is to be changed, it can be removed through an air lock, or the specimen chamber itself is isolated from the column and the specimen stage is removed. If the specimen is not of a metallic nature, it must be coated with a fine-structured metal (usually gold or gold-palladium) that will conduct the electrons. The coated sample is grounded to the stub with silver paint during the mounting of the sample to the stub. The sample and the stub are usually coated together. The chamber is evacuated again to a high vacuum (10^{-3} to 10^{-6} torr) and reopened to the column. The pumping system can be manual or automatic, depending on the instrument.

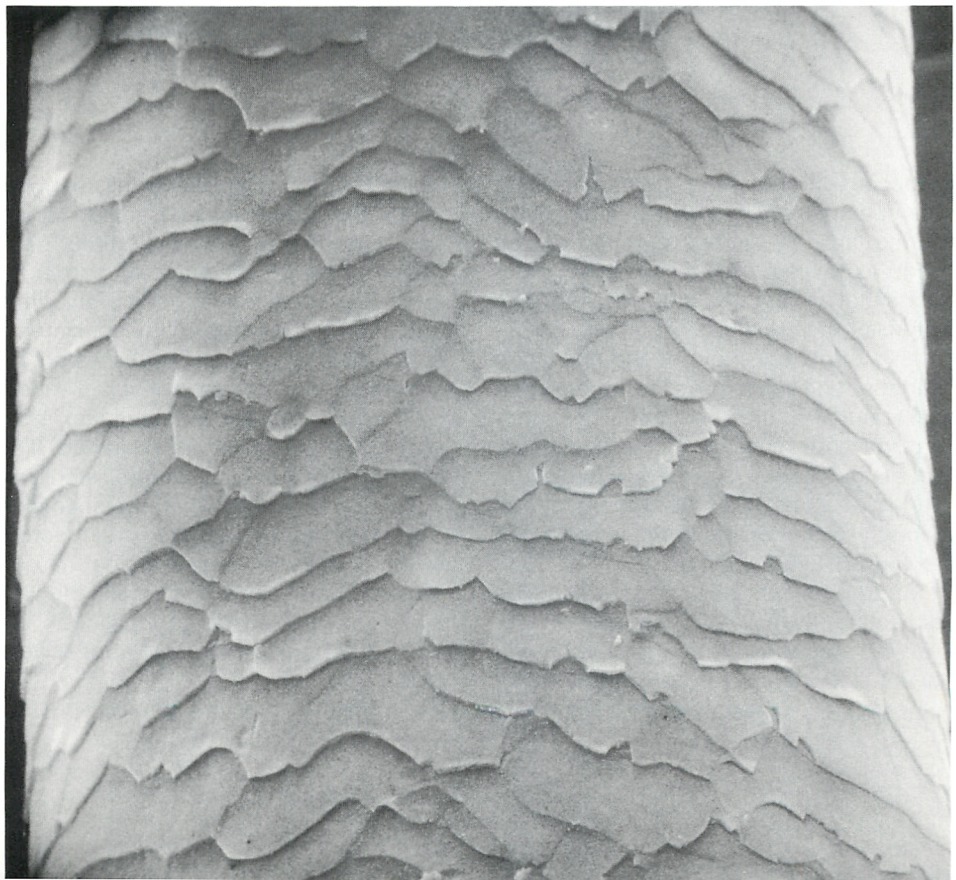
Characteristics of the SEM

The most important characteristics of the SEM are resolving power, depth of field, and contrast. Normally, we would separate these three characteristics of any instrument, but because of unfavorable resolution comparisons with the transmission electron microscope, the widespread acceptance of the scanning electron microscope has been inhibited. An instrument should not be judged by a feature that is limited, while omitting the other features in which the instrument excels. Therefore, depth of field and contrast must be considered along with resolution to give a proper evaluation.

Resolving power in the scanning electron microscope is determined by several factors, such as the finite size of the electron beam (electron gun image), diffusion of the electron beam into the specimen before exciting secondary electron emission, and stabilized lens currents. The size of the beam may be reduced in several ways. Pointed filaments may be used, or a higher kV potential can be employed because the beam is less precise at a lower voltage. For example, at 1 kV, resolution equals 140 nm, while at 30 kV, resolution equals 20 nm (200 A). Raising the voltage also allows the electron beam to penetrate further into the specimen. Finally, the power supply for the lenses must be stabilized to at least one part in 100,000 for high resolution work. With the increased use of solid-state electronics, power supplies are more stable; however, they are still placed away from the column to prevent any interference with the fields of the electromagnetic lenses.

Depth of field, (the region in which all detail is in sharp focus) in the scanning electron microscope is extreme, as illustrated by the SEM micrographs. This

Figure 18—A scanning electron micrograph of a human hair showing the scale pattern on its surface (1500 × magnification).



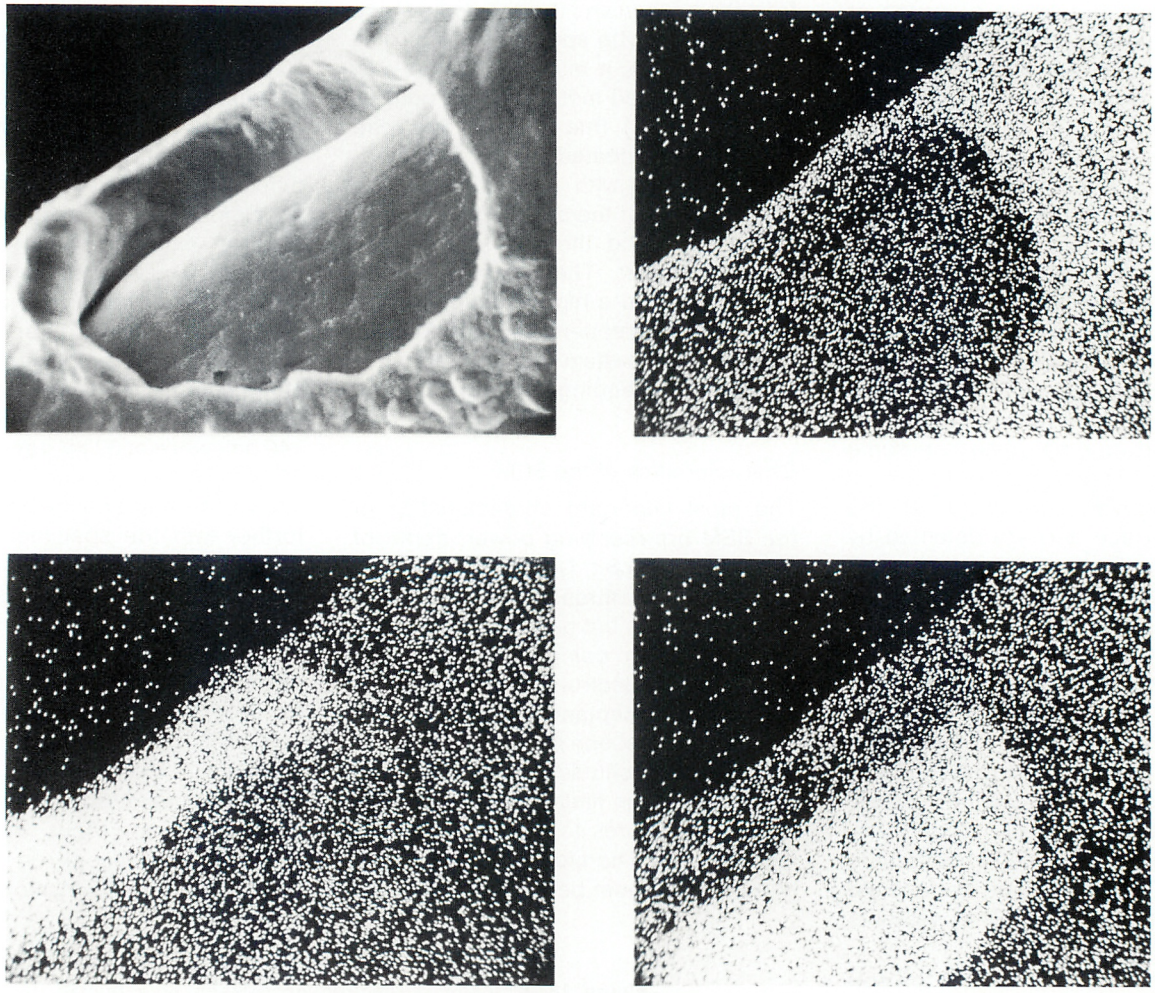


Figure 19—A composite of four illustrations to show different metal locations on the original plated wire (top left). Included are x-ray analysis of copper (top right), nickel (bottom left), and gold (bottom right). (Courtesy of Ultrascan Company.)

is because the electron beam scanning the specimen has relatively little divergence and is almost parallel to the optic axis. Most of the secondary electrons that are emitted are recorded. The intensity of this emission is very sensitive to the tilted angle of the specimen surface, and therefore, the topographical features of the specimen. By changing the area of scan on the specimen, the magnification can be changed in a series of steps (20× to 100,000×; although resolution at 25 nm is restricted to 10,000× to 20,000×).

Contrast is achieved in the SEM by the collecting system. The specimen receives electrons from the scanning beam and generates secondary electrons. The secondary electrons are given off by the specimen and are collected even though they are out of direct sight of the collector. The strong backscattered electrons travel in straight lines and are dependent upon the specimen's shape. Either of these signals can be separated and used; however, the best contrast is achieved when the maximum secondary electrons are collected. The efficiency of the electron emission and collection is highest when the specimen plane is tilted at an angle to the direction of the primary beam and facing the collector. Because this is normally the way an object is seen by an individual, the micrographs from an SEM appear very natural.

Some Applications of the SEM

The electron beam of the SEM may be used as a probe with some modification. It may be used to identify the presence of heavy metals by means of energy dispersive x-ray analysis. By using a single channel analyser, the surface can be mapped to show the location of specific metals (see Figure 19). A special detector and multichannel analyser are needed to collect, separate, and count the emission from the various metals.

Following are a few examples of studies that have been made with the scanning electron microscope: Decomposition of explosive crystals under heat, controlled decomposition of sucrose, crystal growth, ceramic, glass, and ce-

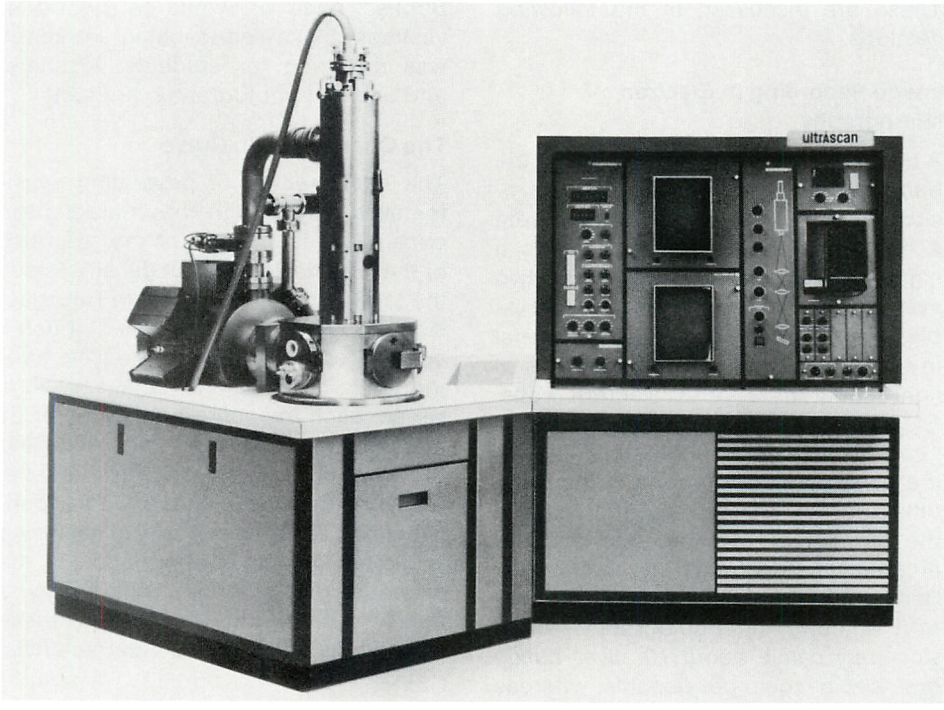


Figure 20—Ultrascan SM-3 Scanning Electron Microscope (courtesy of Ultrascan Company).

ment technology, diamond grinding procedures, growth of oxide material, wear on metal surfaces, heating action on samples, defects in fabrication, and surface studies in general. Also, there are other techniques used with this instrument, and they can be found in *Scanning Electron Microscope* by P. R. Thornton.

The use of the scanning electron microscope will usually produce high-quality micrographs (the film recommended for the photography of the display screen of the scanning electron microscope will be found on page 23). Other requirements for obtaining high-quality micrographs are related to care in sample preparation and the proper use of photographic products related to electron micrography.

Sample Preparation

In the field of electron microscopy, the method of sample preparation determines the final results that will be obtained. If the sample is too thick, improperly stained, poorly shadowed, or contaminated in any way, it may be difficult to evaluate the resulting photographic print. To make the best possible photographs, each step of the technique should be carefully performed, and close attention must be paid to cleanliness. In electron microscopy, cleanliness *is* all-important. All samples should be free of contaminants; any solvents to be used should be of histological or technical quality, and even distilled water should be properly filtered. The preparation area should be free from dust and dirt. Unfiltered air supply systems should be avoided. A "clean" room or roomette with its own filtered air supply, filtered water supply, and highest quality chemicals is a must for applied or experimental research and investigation with the electron microscope.

Another factor, besides sample preparation, that will effect your final illustration is the photography of the image. To give you a better understanding of this subject, we will first present photographic theory as it relates to electron exposures and recording, and second, the practical methods of photography, exposing, processing, and printing.

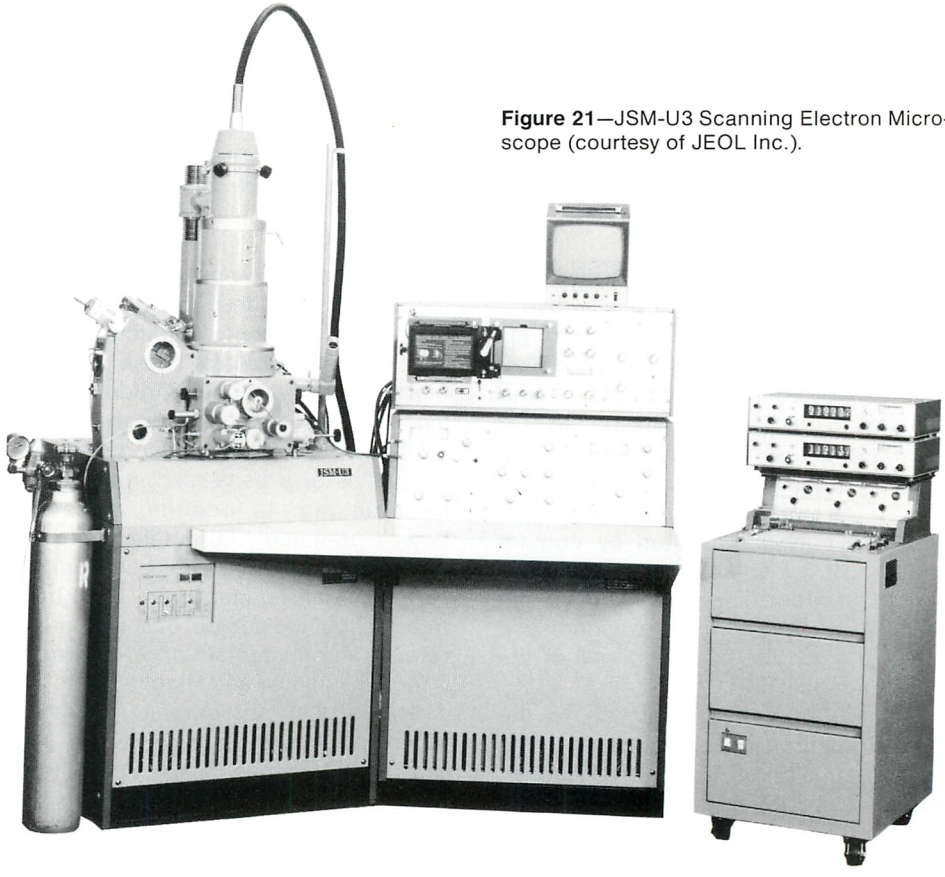


Figure 21—JSM-U3 Scanning Electron Microscope (courtesy of JEOL Inc.).

Photography (Theory)

Photography plays an important role in electron microscopy. It is the final photographic print of a specimen that enables the electron microscopist to communicate his findings and to display his skills as a scientist, artist, and technician. Thus, photography—like the electron microscope itself—is a working tool of the microscopist.

This section is for both the experienced electron microscopist and the newcomer to the field. The experienced microscopist will recognize that much of the information that we have assembled has been previously published in other publications on the subject of electron microscopy. To the newcomer to the field, much of the photographic information probably will be new. Even with some previous photographic experience, he will find that photographic materials respond differently when exposed to electrons rather than photons.

The photographic skills required of the electron microscopists are very diversified. By presenting a brief description of photographic theory, we hope to broaden the general knowledge in this area. Some of the subjects discussed are electron exposure, image recording, and emulsion factors. In the following sections the basic photographic process is reviewed, and in subsequent sections, the application of photography to electron microscopy is discussed.

THE PHOTOGRAPHIC PROCESS

The basic steps in making a conventional photographic recording are to (1) expose the sensitive material, (2) develop the exposed material to yield a negative image of the original subject, (3) rinse, (4) fix the negative, (5) wash, and (6) dry it. If the original subject is to be viewed as a positive print, the processed negative is printed by contact printing or by enlargement onto photographic paper. The exposed print material is developed, fixed, washed, and dried in much the same manner as the original negative.

Much of the theory relating to the photographic process is beyond the scope of this book. There are, however, certain aspects of electron recording and the photographic process that are important to the electron microscopist. These are discussed in the following sections.

Image Recording in Electron Micrography

A parallel was drawn earlier between the basic elements of the electron microscope and the optical microscope. From a photographic standpoint, there is a significant difference between the two systems. The radiant energy responsible for exposing a photographic material in the optical microscope is photons of light equal to 2 or 3 electron volts. Electrons with energy of 50,000 to 100,000 electron volts form the image in the electron microscope. It is this wide difference in energy level that causes the photographic material to respond differently to electrons than to photons. Because of its high energy, *each electron* entering the photographic emulsion may *cause about 10 silver halide grains* to become developable, whereas *10 of the lower energy light quanta (photons) must be absorbed in each grain to have the same effect.* Since the high-energy electron stimulation of a photographic material plays an important role in image formation in electron micrography, a discussion of some of the theoretical aspects of electron recording is given in the following paragraphs. Later, these basic concepts will be related to practical techniques important to the electron microscopist.

Sensitometry

The science of sensitometry is concerned with the description and measurement of the photographic properties of sensitized materials. These principles of sensitometry will be of interest to the electron microscopist. By applying these principles, the speed and contrast characteristics of a sensitized material can be obtained. In addition, resolving power, granularity, and detective quantum efficiency (DQE) of photographic materials can be measured. For further treatment of the subject of sensitometry, refer to *Fundamentals of Photographic Theory* by James and Higgins, or to *The Theory of the Photographic Process* by Mees and James. Both books are listed under "References," page 47. If a basic understanding of photographic sensitometry is desired, Kodak Publication No. Z-22-ED, listed under "KODAK Data Books," page 48, would be a good investment. This self-teaching workbook was designed for students, teachers, and users of photographic products.

The Characteristic Curve

The best method of presenting sensitometric data is with the characteristic curve. This is a graph of the response of the material in terms of density versus the logarithm of the exposure (the classic density/log-exposure curve). Such a curve is obtained by exposing the photographic material to a step-wedge series, each exposure being greater by a constant factor than the preceding exposure. The film is processed and the resultant densities plotted with a densitometer. When the density of each step is plotted against the logarithm of the exposure that produced that density, a curve can be drawn through these points. To produce a characteristic

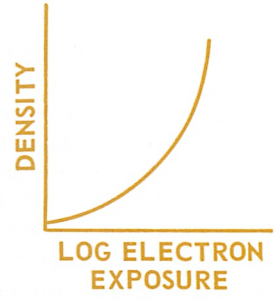


Figure 22—Typical characteristic curve for electron exposure. More exposures will produce a continuing increase in density.

Figure 23—Typical characteristic curve for exposure to white light (photons). The photographic material will increase in density to a certain point and then level off.

curve by electron exposure a step-tablet is not used but instead a stepped exposure series is made on the film or plates. If roll film is used, a separate exposure for each density is made. This film is processed and the density versus log exposure is plotted. The typical characteristic curves are on page 14.

Comparing these two characteristic curves, their shapes are very different. The most important difference between these curves is the photographic *contrast* (which is defined as the slope of the curve). In the photon-exposed curve, the contrast is the same throughout the whole curve up to a point where more exposure does not cause any more density. This is called the *shoulder* and is the upper limit of the straight-line portion of the characteristic curve. However, in the electron-exposed curve there is no linear or straight-line portion. Contrast will continue to increase with increase in exposure until finally it will begin to shoulder off. This is a *very important* aspect of exposures by electrons. With this in mind, recommendations are made to increase exposure time (if specimen stability will permit this) to build contrast into the micrograph.

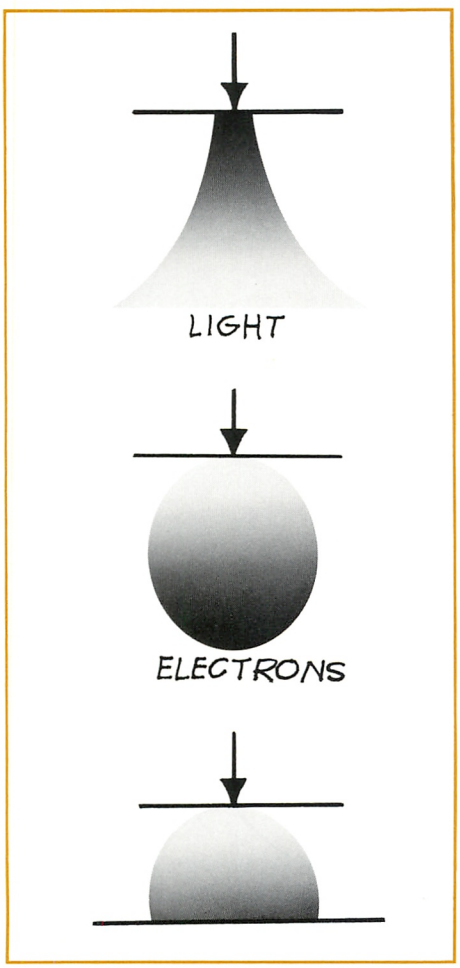


Figure 24—Another difference—electrons must slow down before they can expose the emulsion; therefore, the emulsion must be thick or a limited exposure will result.

EMULSION CHARACTERISTICS AFFECTING IMAGE QUALITY

In this section, we will examine the emulsion characteristics of interest to electron microscopists—contrast, graininess, and resolution. In order to understand and control these important factors, it is helpful to relate them to image formation and development. The photographic scientist prefers to introduce such factors as contrast, gamma, and resolution by first discussing certain basic properties of a recording medium: its spread function, its signal-to-noise characteristics, and its sensitometric curve shape.

From a practical viewpoint, though, it is appropriate to consider the possible *limitations* imposed by certain distinct properties of the photographic material. Some of these limitations are resolving power, spread function, DQE, noise, speed, and graininess.

Resolving Power

The term "resolving power" refers to the ability of an emulsion to record fine detail. If all fluctuations in the electron beam could be eliminated, the limit of information storage in a material would be set by its spread function—a measure of the average projected area over which electron-beam energy is expended.

When electrons enter a photographic emulsion during exposure, some scattering of electrons occurs. The effect is similar to the spreading of a beam of light in a turbid solution, except that the extent of electron scattering is much less. Figure 24 illustrates the energy distribution of a high-energy electron beam in a photographic emulsion. The energy distribution can be determined, as well as its effect on the resolution limit for fine detail in the image.

Spread Function

In practice, an emulsion is capable of resolving two discrete, closely spaced electron beams if they are separated by a distance greater than the spread function of the emulsion. Lower energy electron beams (i.e., less than 50 kV) cause frequent scattering, plus some background scatter, close to the emulsion surface. Higher energy electron beams

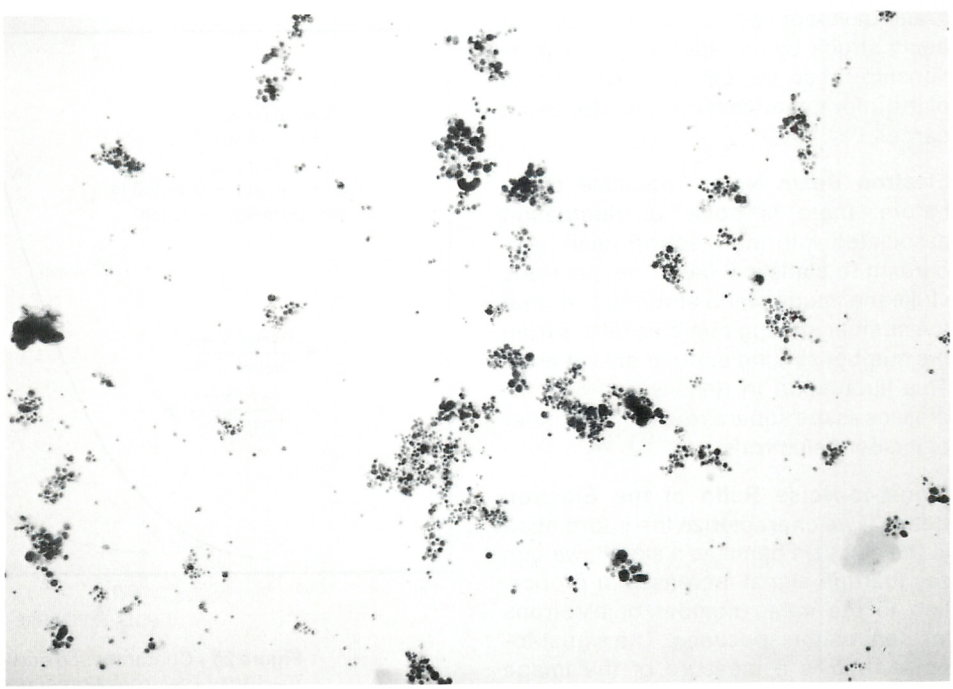


Figure 25—Transmission electron micrograph of extremely fine silver grains used as a test for resolution. Magnification X200,000.

(100 kV), however, penetrate a greater distance into the emulsion before scattering occurs. In the last case, the width of the spread function is greater. Generally, the width of spread functions are slightly less than the electron penetration depth in the emulsion. It is important to note that the spread function is not dependent upon the grain size of the emulsion (as it is for light exposures), but it does depend upon the gelatin content of the emulsion and on the emulsion thickness. For the photographic materials covered in this publication, the spread function is of the order of 5 to 10 micrometers, and the maximum enlargement that provides useful information is about 20×. In practice, granularity limits the information to lower enlargments for all of the materials mentioned in this book, except for KODAK High Resolution Plates.

Detective Quantum Efficiency (DQE)

For the majority of transmission electron microscopes in use today, electron energies are in the 50- to 100-kV range. Each electron may pass through as many as 100 photographic grains, losing enough energy to make some of the silver halide grains developable. The number of grains that develop depends upon the particular developer and the development time used. If at least one grain develops as a consequence of being struck by an electron (a 1:1 relationship), then the emulsion records all of the information that the electron beam carries.

Electron Beam Noise. Because of its nature, there is noise or fluctuation associated with the electron beam. According to statistical laws, the electrons strike the image plane at random so that the number striking one area differs from the number striking another similar area. This fluctuation in density or noise increases as the square root of the number of incident electrons.

Signal-to-Noise Ratio of the Electron Beam. If we characterize the information in the electron beam as a signal, we can say that the signal increases in proportion to the mean number of electrons incident on the specimen. The signal-to-noise ratio is a measure of the image quality of the electron beam and is proportional to the square root of the number of electrons incident on the specimen.

Therefore, the best way to improve the signal-to-noise ratio of the electron image is to collect more electrons from the sample. This can be done in two ways by *either increasing the beam intensity or by lengthening the time of exposure.*

Signal-to Noise Ratio of the Photographic Material. The photographic material also has a signal-to-noise ratio. The signal is an optical density, and the noise is the fluctuation in density from area to area (or the granularity). If the photographic material records all of the signal in the electron beam and does not introduce any noise of its own, it performs perfectly, and the signal-to-noise ratio of the material is identical to that of the original electron beam. However, it is useful to assess the degree to which any photographic material approaches this perfection. For this purpose, a concept known as the Detective Quantum Efficiency (DQE) has been devised.

DQE Defined. Hamilton and Marchant (1967) define DQE as the square of the signal-to-noise ratio in the photographic record divided by the square of the signal-to-noise ratio in the electron beam:

$$DQE = \frac{\left(\frac{\text{Signal}}{\text{Noise}}\right)^2 \text{photographic record}}{\left(\frac{\text{Signal}}{\text{Noise}}\right)^2 \text{electron beam}}$$

A DQE value of 1 indicates a perfect recorder. "DQE = 0.25" means that the signal-to-noise ratio is reduced by half in the recording step.

$$DQE = \frac{(0.5)^2}{(1.0)^2} = \frac{0.25}{1.0} = 0.25$$

Electron Speed

The optical density (at lower densities) of almost any developed photographic emulsion exposed to electrons is merely proportional to the number of incident electrons. Thus, if we plot density (logarithm of the reciprocal of the transmission) versus exposure for a number of materials, we obtain a series of straight lines passing through the origin, each with a different slope. The slope is the measure of the photographic speed of the material to electron exposure, expressed as density units per electron per

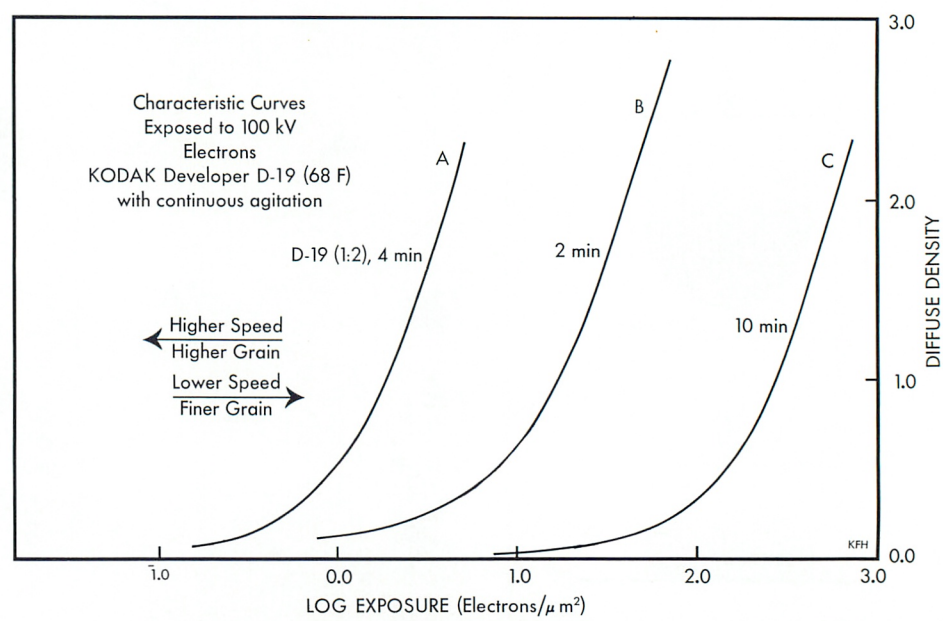


Figure 26—Characteristic curves for emulsions of different speeds.
A—KODAK Electron Microscope Film 4489 (ESTAR Thick Base)
B—EASTMAN Fine Grain Release Positive Film 5302
C—KODAK High Resolution Plate

square micrometer of image. Since the curves are linear and pass through the origin, the slope is numerically given by the reciprocal of the exposure value at a density of 1.0.

The usual way to plot photographic response versus exposure is to plot density versus the *logarithm* of the exposure. All of the characteristic curves shown in this book are plotted in this manner. The photographic speed of a material is normally obtained by taking the reciprocal of the exposure required to produce some fixed density, such as 1.0. This definition of electron speed is expressed in the same manner as most other photographic speed systems.

Electron speed can be used by the microscopist to compare the speeds of products for electron exposure. Higher numbers mean higher speed and less exposure. The system is arithmetic; a material with a speed of 1.0 is twice as sensitive as, and requires half the exposure of, a material with a speed of 0.5. Suppose that you normally use KODAK Electron Image Plates and process them for intermediate speed and grain. You decide that your next specimen will require maximum enlargement. You can then process the plates for minimum grain, estimating the exposure as follows: if accelerating voltage and beam current are held constant, the new exposure will be approximately equal to the old exposure time multiplied by the ratio of the electron speeds. In this case, this ratio is 0.6/0.2; in other words, try an exposure time 3 times as long and modify the processing.

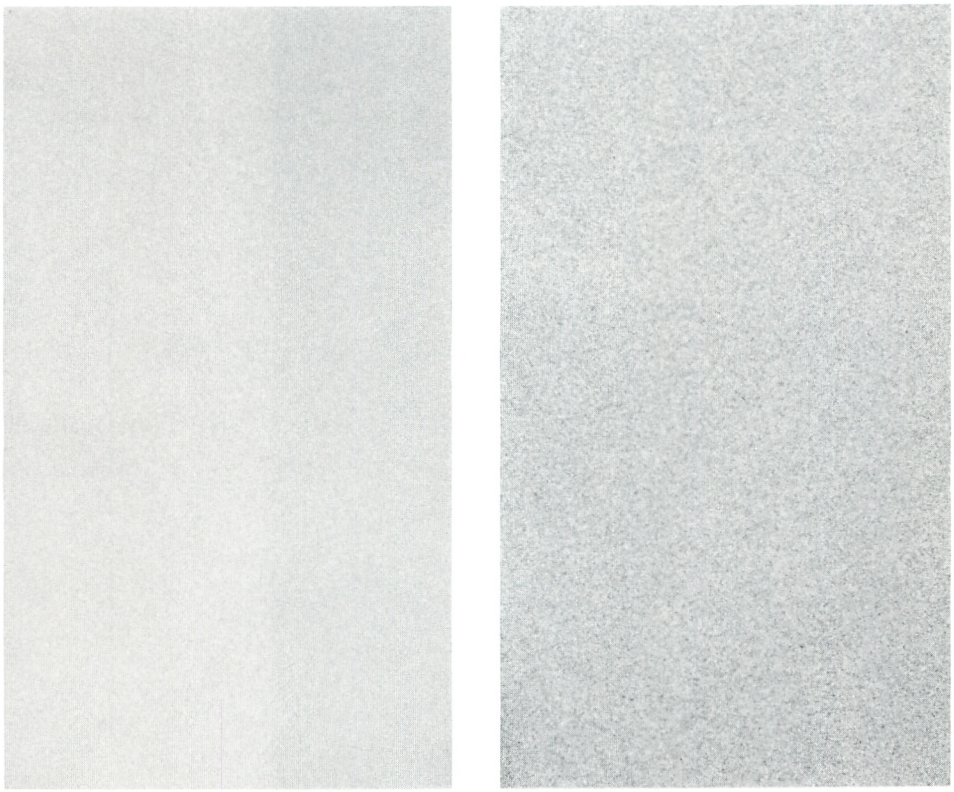


Figure 27—The print on the left illustrates the inherent fine-grain emulsion characteristics of KODAK Electron Image Plate when exposed to light. The print on the right shows the granular structure of the micrograph which results from the *random fluctuation of the high energy electrons.*

Graininess

Whenever a photographic image is viewed under magnification, it does not appear to be homogeneous. In fact, it actually consists of minute particles of silver. These particles are generally referred to as "grains" (see Figure 27). In talking about these grains, the term "graininess" is used and often is mistakenly regarded as a "defect" of the photographic emulsion. Actually, graininess is not a defect.

Graininess is a statistical phenomenon that is several orders larger than the size of grains that form the image. This term is described as *the impression of nonuniformity in the image which is produced on the consciousness of an observer by the granular structure.*

Photographic Grain or Noise?

When a photographic material is exposed to electrons, the granular structure that is produced in the image is much coarser than that produced when the same material is exposed to light. This granular structure is a record of electron fluctuations, or electron noise, in the input beam; *it is not due to the inherent photographic grain of the emulsion.* The RMS Granularity values indicate how electron fluctuation is reduced by increasing exposure and then selecting a suitable development condition to control final image density.

In order to illustrate the difference between electron noise and the inherent grain of the emulsion, a simple test can be conducted. Give one KODAK Electron Image Plate a uniform white-light exposure, using an enlarger as the light source. Expose a second plate in the electron microscope to 100-kV electrons with no specimen in the beam. Develop both plates to a density of about 1.0. (Some adjustment in the exposure of the plates may be necessary in order that both plates have the same density.) Examine the plates visually or under low-power magnification. A comparison of the results clearly illustrates the distinct granular structure that is produced by the random fluctuations of electrons (see Figure 27).

It is important to note that the eye does not actually resolve the individual tracks of the electrons in the emulsion, nor does it resolve the individual photographic grains in the emulsion exposed to white light. Rather, the ob-

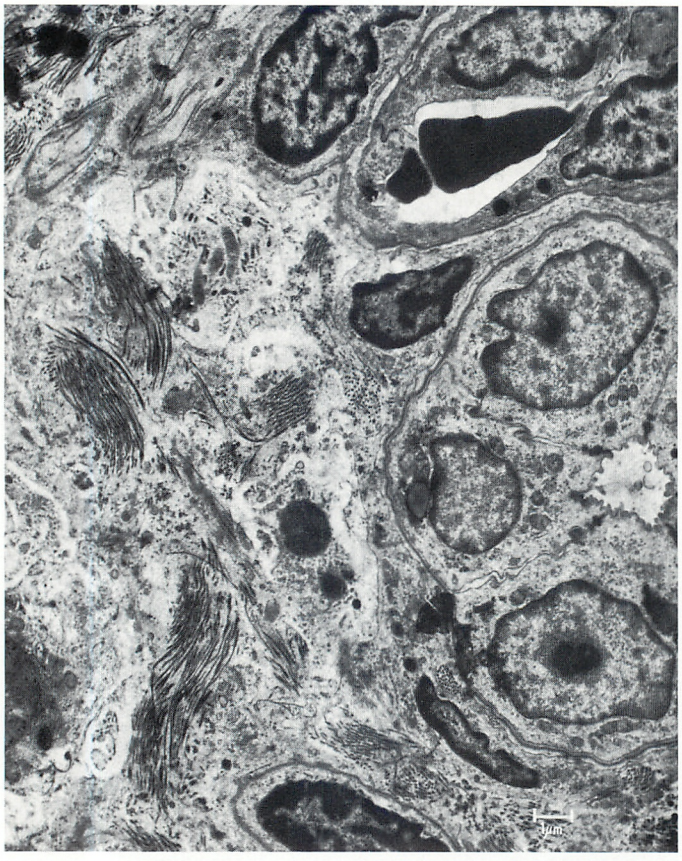


Figure 28—Cross section of animal liver tissue (5000 ×). Contrast was enhanced by staining the tissue with lead acetate. The micrograph was exposed on EASTMAN Fine-Grain Release Positive Film 5302, and printed on KODABROMIDE Paper.

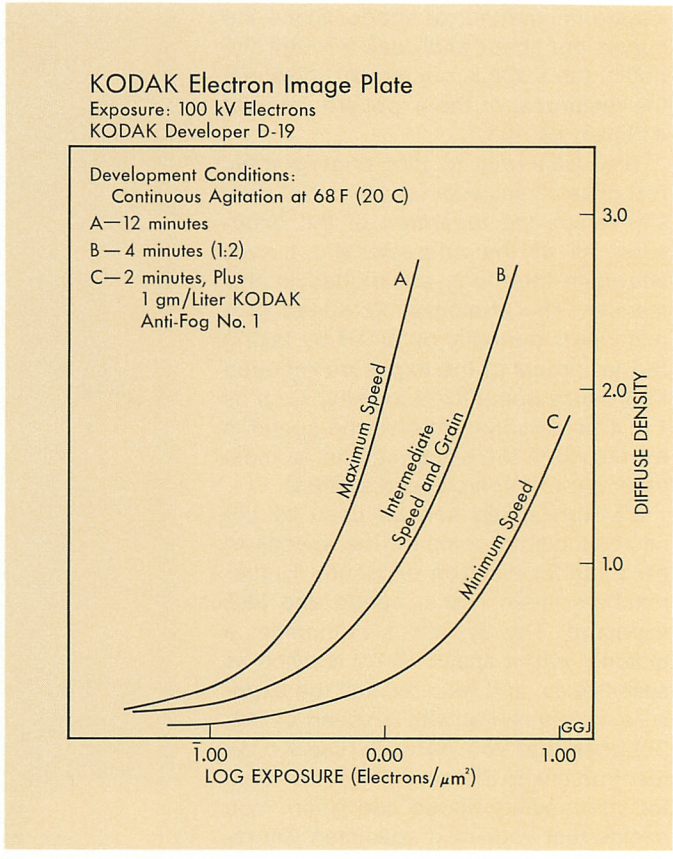


Figure 29—These curves illustrate the change in speed due to a change in development.

server is simply aware of this fluctuation.

Contrast

In electron micrography there are three areas where contrast is discussed. The first is instrument contrast, the second is specimen contrast, and the third is photographic contrast. *Instrument contrast* is achieved by several methods. The use of lower accelerating voltages, the addition of apertures to reduce stray electrons, and the use of special filaments all increase the tonal differences viewed on the fluorescent screen. The *specimen contrast* is related to sample preparation methods where heavy metals and stains are added to the specimen. These add density to certain areas of the specimen and increase the tonal separation. *Photographic contrast* refers to the exposure-density relationship in the photographic material. This material records the specimen contrast and produces the tonal range in the final print.

Tonal range is the total density range of an image. This is simply the difference in density between the lightest and darkest areas. Tonal separation is considered good or adequate if all the desire detail present can be seen. However, if the tonal separation between two areas is poor, detail can be lost. Whatever the tonal separation is, the negative or print material must be able to resolve the fine detail present.

LIMITATIONS IN ELECTRON IMAGERY

Limiting factors are to be expected in most situations, and this is true in image formation in electron micrography as well. The emulsion characteristics of electron speed, detective quantum efficiency (DQE), and graininess are dependent variables; a change in one will effect a change in one or more of the others. Because of this relationship, the practical approach to selecting and using a photographic material is to find the "right" combination of variables associated with the operation of the electron microscope (beam intensity, exposure time, instrument stability, magnification, etc). The challenge for the microscopist is one of stabilizing all variables to achieve the highest quality micrographs.

Maximum Signal-to-Noise Ratio

Since most photographic materials record almost every incident electron, they introduce only a small amount of noise. DQE is, therefore, of the order of 1 for almost all plates and films exposed to electrons. Thus, most photographic materials are about equal in information-detecting ability (for ordinary light exposures, DQE is 0.01 to 0.001).

In order to achieve the highest signal-to-noise ratio in the final record for a given DQE, the material to use is one with the slowest speed so that the largest number of electrons possible can be collected. Conversely, the electron-

beam signal frequently is not sufficiently stable, and more information can be gained by using a higher speed photographic material and collecting electrons over a shorter time. The choice depends upon the particular conditions.

The limits on information detection in electron micrography are set by the signal-to-noise ratio of the electron beam. The signal can be improved by increasing the number of electrons that are allowed to pass through the specimen and be recorded. On this basis we encourage the *longest possible exposure.* With many specimens, however, long exposures cannot be tolerated without introducing image drift or specimen contamination. In these cases, a higher speed photographic material is the best choice. However, this will mean less exposure and, therefore, more noise and appearance of granularity in the final record.

It is important to remember that, for a given exposure time and electron flux at the specimen, the final quality of all micrographs are similar—as long as the DQEs are similar. It doesn't matter whether the micrograph is made by using a fast photographic material, a high instrument magnification, and moderate photographic enlargement, or by using a slower photographic material, a lower instrument magnification, and greater photographic enlargement. If the grain in a print of desired enlargement proves to be unacceptable, the microscopist should use a material with lower electron speed. If graininess is finer than necessary, a faster material can be used.

Process Control for Desired Speed and Grain

In conventional photography, the photographer is accustomed to selecting from a large group of sensitized materials. However, the electron microscopist does not have this wide selection to choose from. Usually the choice consists of one or two photographic materials that have speed-grain characteristics that can be changed by controlling development. With this capability available—especially with KODAK Electron Image Plates—the microscopist can expose the material longer to increase contrast and input signal-to-noise ratio and then can select a processing condition that yields a micrograph of proper density for printing.

The characteristic curves in Figure 29 show how speed can be changed by varying processing conditions with virtually no change in contrast. However, these characteristic curves indicate graphically the reduction in granularity that can be made by the combination of longer exposure and longer development. The curves show that as speed is reduced by development, DQE drops from 0.7 (Process A) to 0.3 (Process C). This difference in terms of final print quality is detectable as a small visual difference. This is the maximum efficiency loss that should be tolerated. It is not recommended that processing modifications be made beyond those shown for Process C.

CONTRAST CONTROL IN ELECTRON MICROSCOPY

Several suggestions follow that pertain to contrast control and how it can be used to achieve the highest possible image quality in the micrograph. Whether they prove useful or not in a given situation will depend on the extent to which the microscopist has already standardized his operating variables. The following suggestions could result in a significant improvement in image quality.

The microscopist has several opportunities to influence contrast control. Some of these opportunities occur in the prephotographic steps—sample preparation methods and instrument methods. Other opportunities are found in processing and printing the electron micrograph. We will discuss the photographic steps that start with final instrument adjustment of beam intensity, magnification selection, and exposure time. Processing the plate or film and printing the resulting negative are the remaining opportunities for influencing contrast control. The following discussion covers the photographic steps of exposing the photographic material and processing the negative. Contrast control in printing is discussed on page 31.

Contrast Enhancement

The biologist working with noncrystalline materials is often faced with a need to boost micrograph contrast. Having taken the proper contrast-enhancing steps in preparing his specimen, his next opportunity for achieving higher contrast is during exposure of the photographic plate or film. The exposure-density relationship for electron-exposed photographic materials offers an opportunity for contrast control both in the electron microscope and during processing. As you remember from earlier discussions, the photographic response obtained from exposure to photons of light and to electrons is different. In conventional photographic techniques, contrast is constant over the straightline portion of the curve. However, with exposure to electrons, the contrast of the micrograph is increased as the density of the negative is increased. Figure 30 illustrates this point.

There is only one way, then, to increase contrast in the electron microscope—increase the density of the electron micrograph.

Here are two practical ways to increase density:

1. Increase exposure with no change in development

2. Increase development activity with no change in exposure

Increasing Exposure. The first way to increase density is to increase exposure. Exposure is increased by using a longer exposure time or by increasing the intensity of the electron beam. As exposure is increased, more electrons are "seen" by the specimen and recorded on the photographic emulsion. Signal-to-noise ratio improves because image contrast increases linearly with exposure, while electron noise (which appears as a grainy pattern) increases less rapidly with the square root of exposure. However, the stability of the specimen (among other considerations) may not permit any increase in exposure time; but if possible, this is the better method to use. An increase in both density and contrast is illustrated in Figure 30.

Increasing Development Activity. The second way to increase density is to increase development activity without changing exposure, as illustrated in Figure 31. Signal and noise increase proportionally, however. The gain in contrast, therefore, does not provide a means for distinguishing weak, fine detail from the noise structure.

Methods for increasing development activity are:

1. Increase development time
2. Increase developer concentration
3. Increase developer-solution temperature

Visual Evaluation of Negative or Plate

All methods for achieving higher contrast result in denser, or darker, micrographs—perhaps darker than you are accustomed to printing or viewing by transmitted light on an illuminator. Even micrographs that appear too dark for good evaluation contain information that is valuable. They can be printed if maximum diffuse density is not greater than 2.5. Remember, *build contrast into the micrograph before you print it.*

We have been discussing the methods of building high contrast into your negative. However, there are some applications where high contrast is not needed. (An example would be photographing electron diffraction patterns where all the diffraction rings should be evident.) In such cases, the density of the film or plate should be kept low. One method of lowering contrast consists of making an unsharp mask and using this mask to make the final print. This lowers the contrast of the large areas with very little effect on the fine detail. Here is an example of how this can be accomplished. Take the KODAK Electron Image Plate bearing the negative and print it *through the glass instead of emulsion-to-emulsion* onto a low-contrast film (such as KODAK Commercial Film 4127). The separation makes the mask unsharp. Process this low-contrast film as recommended. Place the unsharp mask, in contact and in register with the emulsion side of the negative, and then print on a photographic paper of moderate or even high contrast. Much of the gross density difference over the negative is flattened to fit within the range possible in a single print. However, because fine image detail is absent from the unsharp mask, it is not cancelled out. For those microscopists using film instead of plates, the separation distance in making the unsharp mask will have to be determined.

Up to this point, we have given you some basic background information on electron exposure and the recording of this exposure. Now, however, we will turn to the practical applications of photography, including darkroom procedures, exposing, processing, and printing to obtain the final micrograph.

Photography (Practical)

The photographic method of recording a specimen enables the electron microscopist to communicate evidence of his findings and to display his skills. Many times the best methods are not always used to produce a final print. Therefore, to produce the highest quality negative or print, the electron microscopist must be acquainted with what films and plates are available and how to use them properly (exposing, processing, printing, etc).

CHOOSING THE PHOTOGRAPHIC MATERIAL

Earlier, it was stated that virtually any photographic material will record electrons. However, microscopists have narrowed their choice of recording materials, taking into account physical form (plate, sheet film, or roll film) and the best combination of electron speed and granularity for the particular study at hand. With the exception of KODAK Electron Microscope Film 4489 and KODAK Electron Image Plates, sensitized materials used in electron microscopy have been selected from existing products

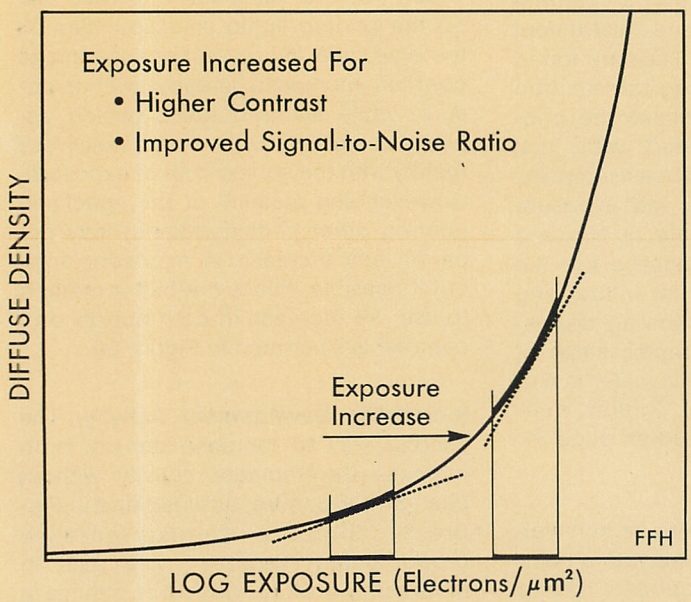


Figure 30—Enhancing contrast and improving signal-to-noise ratio by increasing exposure.

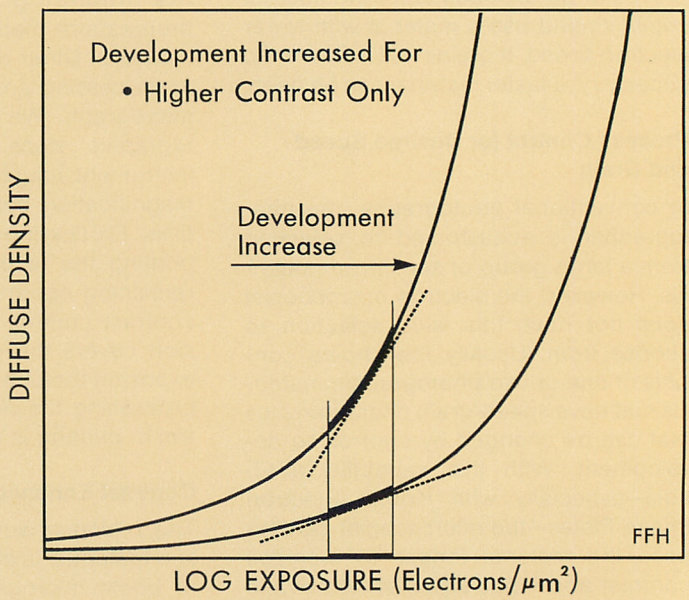


Figure 31—Characteristic curves illustrating higher contrast by increased development.

Electron Micrography

DQE	Recommended Development for Electron Exposure		KODAK Safelight Filter	Ordering Information	
100-kV Electrons	Kodak Developer at 68 F (20 C)	Time		Standard EM Sizes	Minimum Order Quantity
0.7 0.5 0.3	For Maximum Speed: D-19 or HRP (1:2) For Intermediate Speed: D-19 (1:2) or HRP (1:4) Plus 1 gram/l KODAK Anti-Fog No. 1 For Minimum Grain: D-19 Plus 1 gram/l AF No. 1 or HRP (1:4) Plus 5 grams/l KODAK Anti-Fog No. 1	12 min 5 min 4 min 3 min 2 min 3 min	No. 1 (red)	.040-inch glass: 2 x 10 inches 6.5 x 9 cm .050-inch glass: 3¼ x 4 inches 3¼ x 3¼ inches	1 package of 36 plates
0.5 0.5	D-19 (1:2) VERSAMAT Type B Chemicals (in KODAK VERSAMAT Film Processor, Model 5)	4 min 2 ft/min @ 80 F	No. 1A (light red) OA (greenish yellow) OC (light amber)	.007-inch ESTAR Thick Base 2 x 10 inches 3¼ x 4 inches 3¼ x 3 5/16 inches 6.5 x 9 cm 7 x 7 cm	1 package of 100 sheets
	D-19 HRP (1:4)	8 min 5 min	No. 1 (red)	4 x 5 inch (sheet)	1 package of 50 sheets
scope," by M. M. Salpeter and L. Brachmann, *The Journal of Cell Biology*, Vol. 22, No. 2 (1964), pp. 469-77.			No. 1A (light red)	4-ounce bottle of liquid emulsion	1 bottle
0.5	D-19	2 min	No. 1A (light red)	35mm x 150 feet 70mm x 150 feet	See Graphic Arts or EM Suppliers
0.3 0.3	D-19 DEKTOL	2 min 2 min	OA (greenish yellow)	35mm x 100 feet 70mm x 100 feet	1 roll See EM Suppliers
—	D-19	10 min	No. 1A (light red) OC (light amber)	.040-inch glass: 2 x 10 inches 6.5 x 9 cm .050-inch glass: 3¼ x 4 inches 3¼ x 3¼ inches	No. of 36-Plate Packages (1 Case) 15 packages 12 packages 12 packages 12 packages
0.7	D-19	3 min	No. 2 (dark red)	.060-inch glass: 2 x 10 inches	1 package of 36 plates
0.9 1.0	D-19 D-8, or D-19	3 min 3 min 20 min			

moisture. When the instrument reaches the proper vacuum, the filament can be turned on and the exposure made.

How is exposure calculated on the electron microscope? The method of calculating the amount of exposure necessary to give a good negative is different for each instrument; some instruments have photometers, while others have fully automatic exposure methods. The instruction book provided with the electron microscope will usually present a step-by-step procedure for properly exposing negatives on film or plates. One method that works well is to set up a small meter that has a voltage control switch attached. The meter consists of a fluorescent material that resembles the color of the screen of the microscope. At a known current, the meter gives a certain brightness that visually matches the fluorescent screen of the electron microscope.

One method of determining the proper exposure level that has been used with success with microscopes at Eastman Kodak Company is as follows: a 4-inch strip of illuminescent material is taped to one side of the screen (outside the vacuum). This material, when an AC voltage is applied, will glow a color very similar to the phosphor of the fluorescent screen. The brightness of the strip of material is matched to the screen, the setting on a small AC variable transformer is recorded, and an exposure series is made. Similar exposure series are made for other screen brightness levels. These exposure series are processed and the best exposures are selected. Additionally, these values can be plotted on a graph. If a low electron intensity is necessary to prevent specimen damage, thermal drift, etc, a low setting on the variable transformer with a corresponding long exposure would be a necessary choice. For normal work, a medium setting and a 4-second exposure would be suggested.

The material used was 1'' x 4'' Electro Illuminescent Strip No. 94-0030-1G Type 5, 120 volts, 60 cycle, and is available from Grimes Manufacturing Company, Urbana, Ohio 43078.

PROCESSING THE NEGATIVE (FILM AND PLATES)

After the proper exposures have been made, the films or plates are removed from the electron microscope and are processed under the proper safelight. If the proper development conditions are selected, KODAK Electron Image Plates can be processed for maximum, intermediate, or minimum speed. This versatility allows the microscopist to obtain high-quality micrographs for a variety of samples and instrument conditions. These development recommendations are offered as a guide. If desired, developer conditions can be slightly modified to achieve speed-grain characteristics intermediate to those indicated.

Table 3 Developing KODAK Films and Plates

Plates and Films	KODAK Developers* at 20 C (68 F)	Time (minutes)
KODAK Electron Image Plate	For Maximum Speed:	
	D-19 or	12
	HRP (1:2)	5
	For Intermediate Speed:	
	D-19 (1:2) or	4
	HRP (1:4) plus 1 gm/l KODAK Anti-Fog No. 1†	3
	For Minimum Speed:	
	D-19 plus 1 gm/l KODAK Anti-Fog No. 1 or	2
	HRP (1:4) plus 5 gm/l KODAK Anti-Fog No. 1†	3
KODAK Electron Microscope Film 4489 (ESTAR Thick Base)	D-19 (1:2) or	4
	VERSAMAT Type B Chemicals (in VERSAMAT Film Processor, Model 5)	2 ft/min @ 80 F
EASTMAN Fine Grain Release Positive Film 5302	D-19	2
	DEKTOL	2
KODALITH LR Film 2572 (ESTAR Base)	D-19	2
KODAK High Resolution Plates	D-19	10
KODAK Nuclear Track Plates, Type NTB, or Type NTB2	D-19	3
	D-19 or	20
	D-8	3

*The plates and films were processed in tanks, with nitrogen-burst agitation during development (1-second bursts at 8-second intervals). This method of development is strongly recommended, since it provides the most desirable conditions for uniform development and yields the most reproducible results. Regardless of the equipment used, the suggested developer concentrations and development times shown serve as starting points. Use of the proper safelight is recommended (see Table 2).

†See page 27 for method of mixing stock solution of KODAK Anti-Fog, No. 1.

Notes on Processing Steps

1. *Develop* plates and film under the recommended safelight conditions with continuous agitation for the time indicated in Table 3.

2. *Rinse* in running water for 1½ minutes at 18 to 21 C (65 to 70 F). Agitate continuously by lifting and reimmersing in the rinse tank or tray. *Do not use conventional stop bath solutions because they may produce a mottled appearance on plates or films used for electron exposures.*

NOTE: The *rinse* step for plates and film is *very important*. Failure to rinse in water for a full 1½ minutes with good agitation can cause mottle, unevenness, or stain, due to excessive retention of developer in the emulsion.

3. *Fix* with frequent agitation. Films and

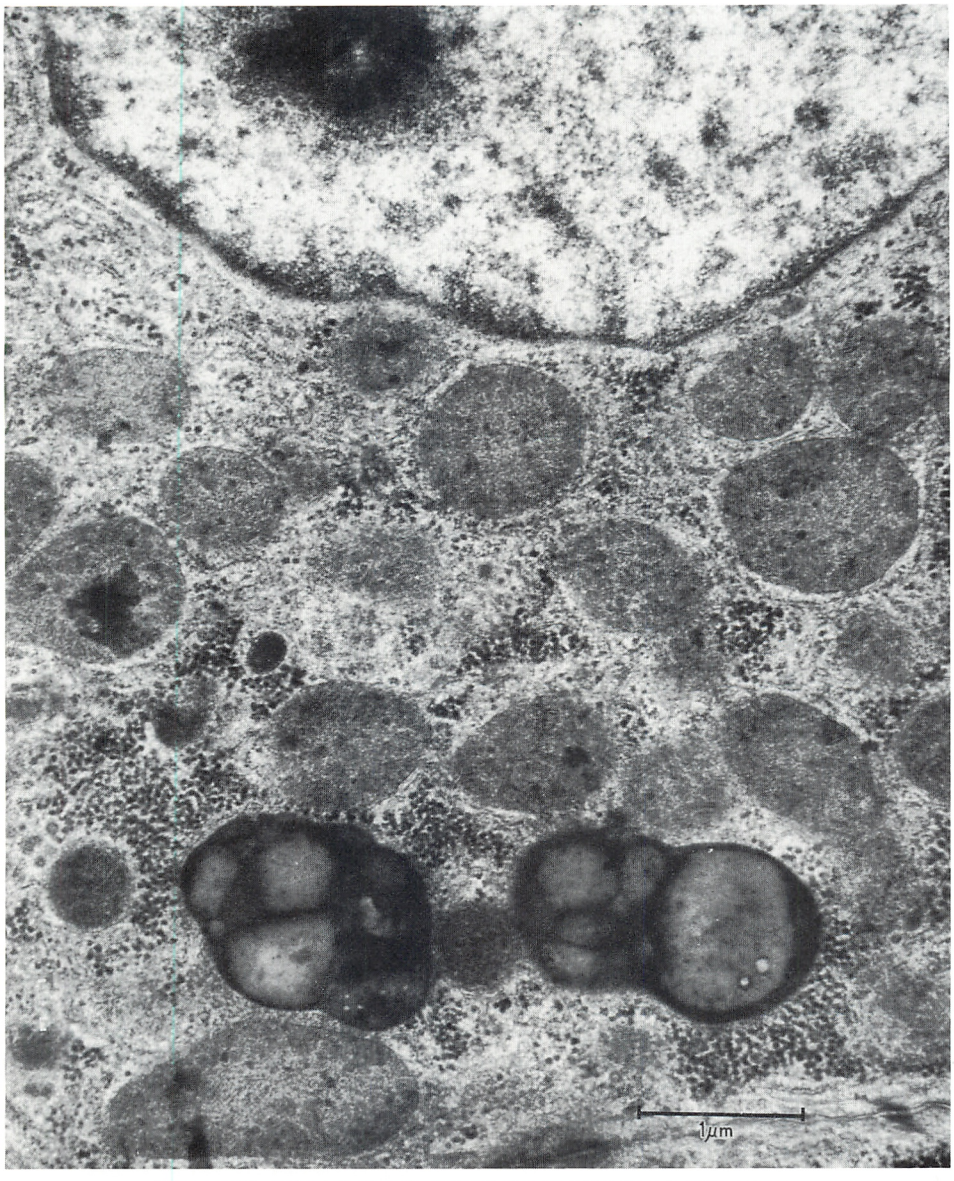


Figure 35—Fat globules in animal tissue (22,000×). This micrograph was exposed on KODAK Electron Microscope Film 4489 and printed on KODABROMIDE Paper F-2.

plates are properly fixed after they have been immersed in the fixer for twice the time taken for the milky appearance to clear completely. Clearing time may vary for emulsions of different thicknesses. After the emulsion has cleared, white lights may be used.

NOTE: KODAK Rapid Fixer is not recommended for nuclear emulsions. This fixer may cause a fading of the image. Use KODAK Fixer for the recommended time.

4. *Wash* for 30 minutes in running water, sufficient to give one complete change of water every five minutes. To minimize drying marks, rinse one minute in KODAK PHOTO-FLO Solution after washing, or wipe surfaces carefully with a KODAK Photo Chamois or soft viscose sponge.

Rapid Wash. KODAK Hypo Clearing Agent can be used *after fixing* to reduce washing time and to conserve water. Remove excess fixer (hypo) by rinsing in water for 30 seconds; then bathe film or plates in KODAK Hypo Clearing Agent for 2 minutes with moderate agitation. Wash for 5 minutes in a water flow sufficient to give at least one complete change of water during the 5-minute wash period. Rinse for 1 minute in KODAK PHOTO-FLO Solution to promote uniform drying.

5. *Dry* in a dust-free area or in a dryer with normal relative humidity. To accelerate drying, use a heated cabinet or a current of warm air. Avoid excessive heat; a temperature of 38 C (100 F) is recommended.

Besides exposure, four main considerations in development govern the density, contrast, and uniformity of the negative. These factors are the type of developer used, the developer temperature, the length of time of development, and the agitation given during development.

Development: Sheet film or plates can be processed in a tray or tank. Although tray development is quite satisfactory, it is recommended only for small amounts of films or plates. Generally, tank development yields more consistent results where considerable quantities of negatives are involved. This consistency is achieved only by paying careful attention to agitation and temperature control.

Short lengths of roll film are processed in spiral reels, either singly or in batches. For consistent quality, use fresh developer each time a new roll is processed; maintain the proper temperature and agitation.

Three of the development conditions shown in Table 3 require the use of KODAK Anti-Fog, No. 1 (benzotriazole). The most convenient method for adding the required amounts of this material to *freshly prepared* developer solutions is to use a stock solution of Anti-Fog No. 1 in ethylene glycol. (Benzotriazole is only slightly soluble in water or developer solution.) To prepare and use a stock solution, proceed as follows:

KODAK Anti-Fog, No. 1, Stock Solution

Ethylene Glycol (high purity) . . 700.0 ml

KODAK Anti-Fog, No. 1 benzotriazole *in powder form** . . . . . 166.7 grams

**Do not* use the tablet form. Each tablet contains only 0.03 gram of benzotriazole and, therefore, for this solution alone, over 5500 tablets (110 50-tablet bottles) would be needed.

Table 4 Processing Steps for KODAK Films and Plates

Processing Step	Chemical	Time (minutes)	Temperature	Remarks
1. Develop	See Table 3 on page 26 for choice of developers and recommended times			
2. Rinse	Water	1½	18-21 C (65-70 F)	Use continuous agitation
3. Fix	KODAK Fixer or KODAK Rapid Fixer	10 5	18-21 C (65-70 F)	Use intermittent agitation
4. Wash or Rapid Wash	Water KODAK Hypo Clearing Agent Water	30 2 5	18-21 C (65-70 F) 18-21 C (65-70 F) 18-21 C (65-70 F)	Use moderate agitation Use moderate agitation Complete change of water required during wash period
	KODAK PHOTO-FLO	1	18-21 C (65-70 F)	
5. Dry				Area must be dust-free

Warm the ethylene glycol to 49 C (120 F), add the Anti-Fog No. 1, and stir until completely dissolved. Cool to 24 C (75 F), add ethylene glycol to make a total volume of 1 liter, and stir until the solution is uniform. If the stock solution is stored in a stoppered brown bottle, it may be kept indefinitely.

For each gram of KODAK Anti-Fog, No. 1 required, use 6 ml of the prepared stock solution.

Tank Development (With Intermittent Nitrogen-Burst Agitation): With the nitrogen-burst agitation unit set for about 1-second bursts at 8-second intervals, start the timer. Smoothly and carefully lower the hangers or rack into the developer solution. Immediately tap the hangers sharply two or three times on the upper edge of the tank to dislodge any air bells clinging to the emulsion surface, and check the spacing of the hangers to make certain that they are at least ½ inch apart. If a rack is used, rapidly move the submerged rack back and forth several times. In addition to dislodging any air bells, this action prevents the emulsion side from adhering to the sides of the slots in the rack.

Allow the plates or film to remain undisturbed (except by the automatic agitation from the nitrogen bursts) until development is complete. Lift the hangers or rack from the developer. Allow excess developer to drain for 1 or 2 seconds, and then transfer the rack or hangers to the next tank.

NOTE: The pressure of the nitrogen gas should be sufficient to produce with each burst a rise of about ⅝ inch in the level of the developer solution. It is this rise in solution level, not the bubble action, which provides the agitation necessary for uniform development. Kodak Pamphlet No. E-57, *Gaseous-Burst Agitation in Processing,* describes in detail equipment for gaseous-burst agitation in tank processing, as well as installation and operation of the equipment. The KODAK Intermittent Gaseous Burst Valve, Model 90B, which is described in Pamphlet No. E-57, can supply a controlled, intermittent gaseous burst for agitation of any photographic processing solution. The Gaseous Burst Valve, Model 90B (plus a suitable gas distributor for the developer tank and related tubing and fittings), can be easily installed.

Tank Development (Without Nitrogen-Burst Agitation): Start the timer. Smoothly and carefully lower the plates or films as a unit into the developer. Space hangers ½ inch apart. Immediately tap the hangers or rack sharply two or three times on the upper edge of the tank to dislodge any air bells clinging to the emulsion surface.

Allow the hangers to remain undisturbed in the developer for about 20 seconds. Then, quickly but smoothly, lift them clear of the developer solution. Keeping the plane of the plates or films vertical, tilt them one-quarter turn, either clockwise or counterclockwise, and back. Immediately reimmerse them, and again lift them from the solution, tilting them one-quarter turn in the opposite direction. Quickly reimmerse them in the solution and check the spacing of the hangers to make certain that they are ½ inch apart. It is important that the hangers or rack be tilted as close as possible to 90 degrees. The entire cycle of lifting and reimmersion should be as rapid as possible without interfering with smooth operation. It should be completed in about 5 to 7 seconds (see Figure 36).

When development is complete, lift the hangers or rack from the developer, drain them for one or two seconds, and transfer them to the next tank.

Tray Development (For one or several plates or films): Generally, the electron microscopist has several plates or films to process at a time and equips his darkroom for more efficient processing with tanks. However, in the event that one or several plates or sheets of film are to be processed in a tray, the following procedures can be used.

To process, select a tray slightly larger than the plate or film and fill the tray with developer to a depth of ½ to ¾ inch. (A tray with a ribbed bottom is suggested, in order to provide a good flow of developer beneath the plate or film for complete removal of the dyed-gelatin backing.)

Start the timer and immediately slide the plate or film, emulsion side up, smoothly into the developer. Agitate the tray continuously throughout the devel-

opment as follows: Raise the left side of the tray about ½ to ¾ inch; lower it smoothly, and then immediately raise and lower the near side in the same way; next, raise and lower the right side; again raise and lower the near side. These four maneuvers constitute an "agitation cycle"; it requires a total time of about 8 seconds. For development times of 5 minutes or less, continuous agitation is recommended. For development times over 5 minutes, the agitation cycle should be repeated several times per minute. *The plate or film should remain completely immersed in the developer during the agitation cycle and for the full development time.*

To process several plates or films, select a tray large enough to hold them in separate positions. Enough developer should be provided to cover them at all times as the tray is rocked.

Start the timer. Then, handling each plate or film individually by the edges, slide it (emulsion side up) into position in the tray so that the entire emulsion surface is wetted almost instantly. Note the position of the plates or films so that they can be removed in the same order in which they are immersed. With a large wad of cotton which has been previously soaked in the developer, swab lightly over the emulsion surface to remove any air bells. Agitate the tray continously throughout development (except when the development time exceeds 5 minutes) by following the same procedure outlined for a single plate of film. Be sure that they do not overlap during the agitation cycle.

Small Tank Development (Roll film): Load the film on the reels, being careful not to scratch or touch the emulsion. Start the timer. Place the loaded film reel or apron into the tank containing the developer. Tap the reel or apron gently on the bottom of the tank to dislodge air bells. Now, replace the tank cover. The rest of the process can be carried out in normal room light.

After the film has been immersed for 30 seconds, agitate the tank for 5 seconds. Agitate by sliding the tank back and forth over a distance of about 10 inches. Rotate the tank about one-half turn at the same time, or, if the tank can be turned upside down without spilling the solution, invert the tank once per second during the agitation interval. Repeat this agitation at intervals of 30 seconds for the remainder of the development time.

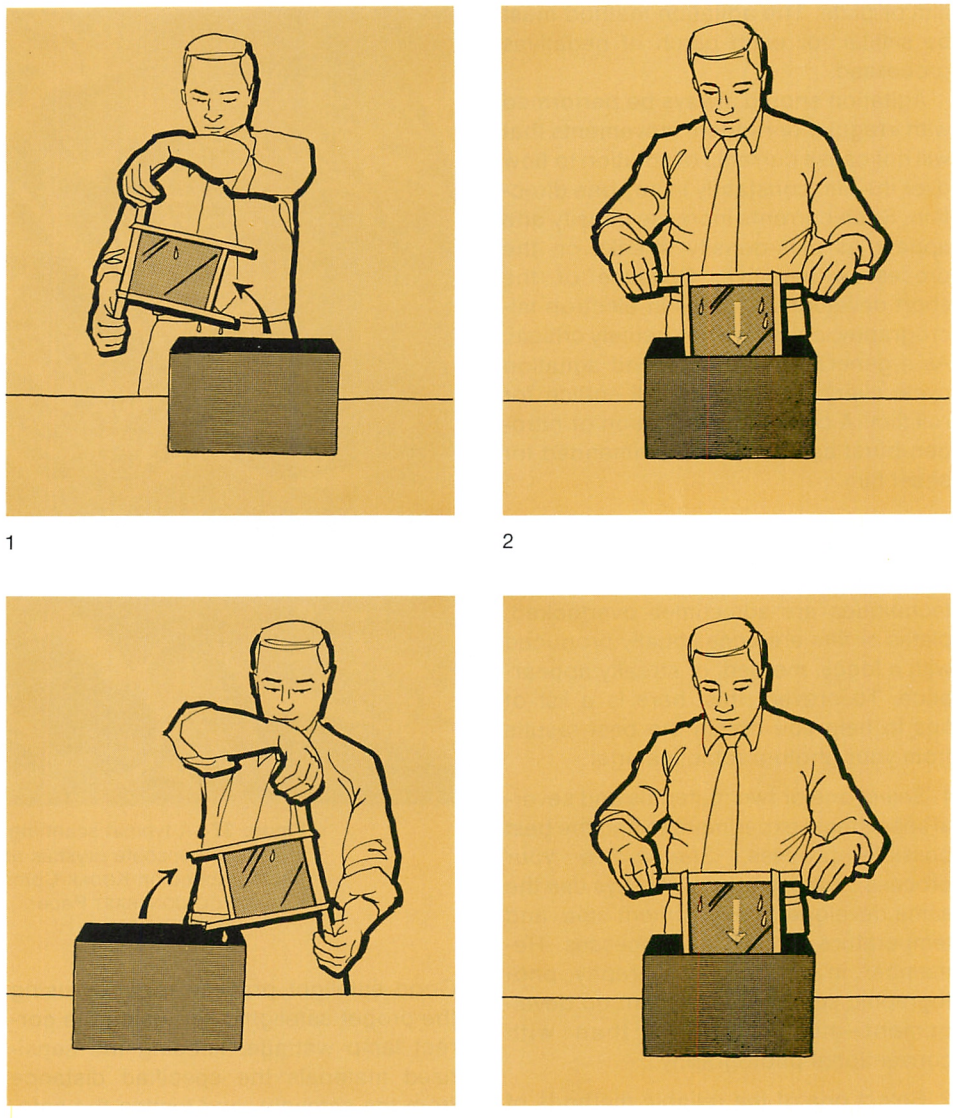


Figure 36—For uniform processing of plates or films, follow this four-step agitation cycle. Each cycle should be completed in about 5 to 7 seconds. Use this same agitation cycle for processing plates in a rack.

Machine Processing: In many electron-microscope installations, the volume of plates and films that are processed warrants installation of a processing machine. Such machines provide either automatic or semiautomatic processing for the commonly used EM plate and film sizes. Accurate time and temperature controls are provided. Efficient agitation with controlled nitrogen burst is incorporated in most units to provide uniform, reproducible processing conditions. We suggest that you contact EM suppliers to find out where such equipment is available.

Agitation: The purpose of agitation is to remove the by-products of development from the surface of the emulsion, and to allow fresh developer to act upon the exposed areas. Since agitation affects the rate of development, consistent quality is achieved only when agitation is uniform over the entire surface of the

film or plate. The agitation method must be similar for each batch of negatives processed.

Agitation should always be performed with irregular or random movements that will not cause currents of solution to flow over the film constantly in any one direction. Such currents increase density and appear as plus-density streaks on the processed negative. Because of the short development times in electron micrography, agitation is extremely critical. As a general rule, a 5-second agitation cycle every 30 seconds will suffice for roll film. A continuous agitation or nitrogen-burst agitation is recommended for sheet film.

Processing and Handling Tips

Because good processing and handling techniques are sometimes overlooked, plates or film are occasionally produced with a foggy, mottled, or streaky appearance. To prevent this, here is a list of tips to help you obtain the best results from your photographic material:

1. Select one or two materials and several specimens; experiment to find the best possible process. *Standardize* your process on these results. Always use the same developer, development time, and temperature for similar processes. Remember, in electron micrography, photographic speed is dependent on development—much more so than with conventional photography.

2. Select one of the reliable methods of agitation and *always* use it. Use consistent agitation for every plate or film and for each process. This is the key to reproducible results and quality. The best agitation techniques for small laboratories are nitrogen-burst agitation, agitation in a small tank, or continuous manual agitation.

3. Use a water rinse after development, adequate fixing (never less than twice the time required for the emulsion to clear), and proper washing.

4. Use fresh processing solutions. Adopt a regular schedule of solution replacement, based on the quantity of materials processed or on the time the solutions have been in use.

5. Check Table 2 or the label on the photographic material for the proper safelight recommendation—then follow it. Good safelight practice requires using the proper safelight filter, using the correct lamp wattage, keeping the sensitized materials the specified distance from the safelight, and testing the safelight occasionally. Test the safelight by leaving a normally exposed plate or piece of film (half-covered) on the darkroom counter for 30 minutes with the safelight on. Process the plate or film and examine the two halves for any evidence of fogging. Similar densities on both indicate a properly adjusted safelight.

6. Keep the darkroom, the processing tanks, and the mixing vessels clean.

Figure 37—A typical scanning electron micrograph. The subject is sodium benzoate crystals at 500× magnification. These were photographed on KODAK Commercial Film 4127 and enlarged on KODAK POLYCONTRAST Paper.

PRINTING THE ELECTRON MICROGRAPH

In a real sense, it is the electron micrograph that is the final result of the scientist's, biologist's, and metallurgist's work. It is his form of advertising, and is a direct indication of his labors and skills. This electron micrograph should be carefully selected so that it is truly indicative of the contents of the publication or report, and properly illustrates the text. When the best printing methods are used, the finished work will be rewarding both to the microscopist and to the reader.

Printing the micrograph negative properly is the final step in displaying all the work that has been done previously (specimen selection, preparation, exposure, and processing). If the negative exposures have been standardized (similar density, illumination, background, etc), the printing procedure can be easily performed or can even be sent to a photographic department or a commercial printing service. Many times, the printing operation is carried out by a technician who may be unaware of what the micrograph should show. However, the final approval of the print should always be the responsibility of the microscopist. He determines what the print should illustrate. To prevent delay and poor-quality prints, the printing procedures must be followed closely with spe-

cial attention paid to handling, safelights, cleanliness, etc. A proper combination of enlarger, photographic paper, and developing procedures must be followed to be sure that any detail present in the negative is not degraded in the printing procedure. The following comments on printing are intended as a guide to producing good micrographs.

Choice of Enlargers

The enlarger (and the manner in which it is used and maintained) plays an important role in producing high-quality, high-definition prints. Because there are many types of enlargers available today, it is important to select one that fits all your requirements. A diffusion-type enlarger is *not* recommended for electron micrography or other scientific applications, because this type of enlarger does not give the sharpness and contrast required to reproduce the fine detail in an electron micrograph negative. A conventional, good-quality condenser enlarger equipped with an ordinary enlarger lamp and a double-condenser lens represents the *minimum* requirements for good negative reproduction.

For maximum resolution in printing electron micrographs, use a point-light source enlarger with an effective condenser-lens system. This enlarging system provides the best method of reproducing the image and fine detail in the negative. For all critical work, this type of enlarger should be used. A list of several enlarger suppliers can be found on page 47.

All enlargers should be equipped with a *voltage control* to prevent fluctuation in the line voltage. Exposures in printing can be affected considerably by variations in the voltage of the electric current applied to the printer or enlarger lamp. Light output may drop as much as 30 percent with 10 percent drop in voltage. Also, when the voltage changes, so does the color temperature of the light source. As voltage drops, the light becomes yellow, a color to which photographic printing papers are less sensitive. Sudden changes in voltage are usually due to switching equipment on or off, such as studio lights and drying machines. The current applied to an enlarger lamp can be regulated by use of a constant-voltage transformer or voltage regulator.

Care of the Enlarger. Advantages gained by using the best enlarger can be lost if proper care and maintenance of the enlarger is ignored. Take the following precautions:

1. Keep the lens and condenser surfaces free of dust and fingerprints. Any accumulation of dust particles on the optical elements will diffuse the light source and thus reduce contrast and sharpness of the image.

2. Be sure the enlarger is firmly positioned and isolated from sources of vibration.

3. Use a focusing magnifier, for example 25X, to focus the image on the enlarger easel. The use of the unaided eye for fine focusing may not yield the finest detail.

4. Use the proper negative holder for making the exposure on photographic paper. Be sure this holder is clean and holds the negative sufficiently flat.

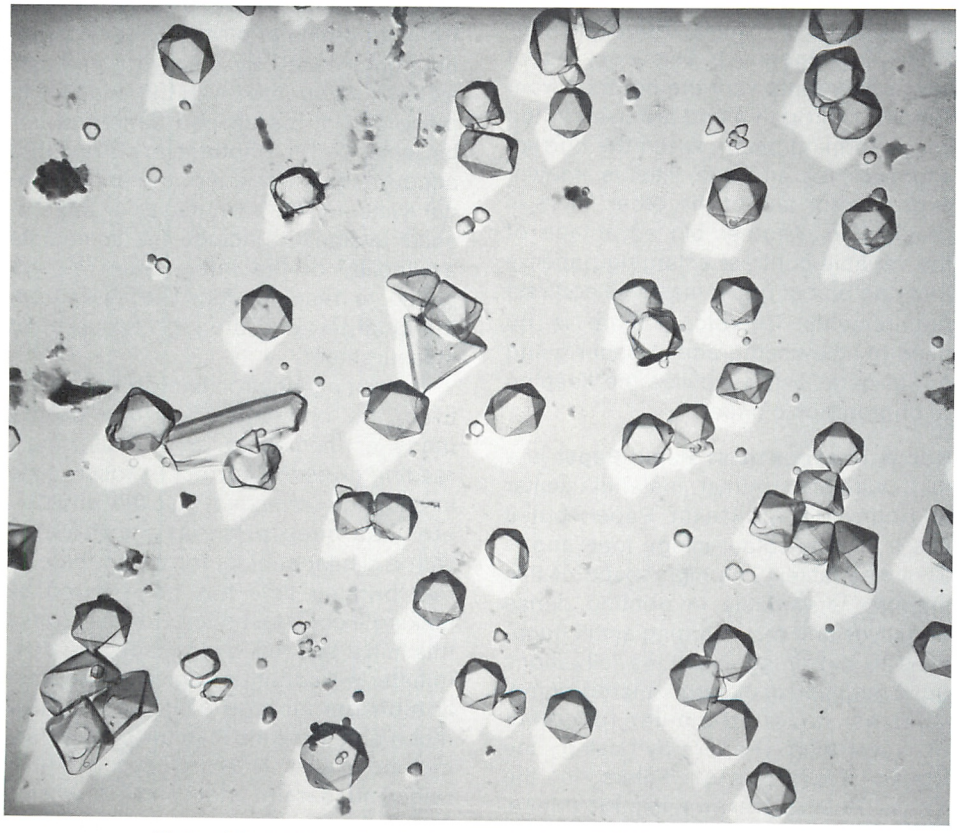


Figure 38—Ag Br grains shadowed at 1:1 or 45° (the height of the grain equals the length of the shadow). Transmission electron micrograph at 20,000 × magnification.

Choice of Paper Grade

Kodak photographic papers used by electron microscopists are of two types: the type that is processed by the familiar tray-processing method, and the type especially designed for rapid, machine stabilization processing. (A complete discussion of all aspects of stabilization processing begins on page 38.)

Contrast. The contrast capacity of a photographic paper is a built-in feature, so there is very little that can be done to change it by making adjustments to the developer composition or changing development times, except within very narrow limits. *Therefore, best results will be obtained by selecting the proper paper grade to print the density scale of the negative, and by processing the paper as recommended.* Generally, maximum contrast for a given paper grade/developer combination will result if the printing exposure is adjusted so that full

Paper-Grade Number	Scale Index	Density Scale of Negative	Contrast of Negative
1	1.5	1.2 or higher	High
2	1.3	1.0 to 1.2	Normal
3	1.1	0.8 to 1.0	Low
4	0.9	0.6 to 0.8	Very Low
5	0.7	0.6 or lower	Extremely Low

development can be carried out. Underdevelopment of an overexposed print reduces the contrast capability for the particular paper grade.

Density Scale. Most of the time, a darkroom technician can correctly choose the desired paper grade. But now and then, a judgment must be made regarding the correct choice of paper for an obviously contrasty or flat negative. A photometer or densitometer can probably "guess" better than a human (with savings in time and material).

The density scale of the negative to be printed can help you choose the right grade of paper. To determine the density scale of the negative, read and record the shadow density and the highlight density. The shadow density is the lightest area of the negative, while the highlight density is the darkest area of the negative where detail is present. The difference between these is the density scale of the negative. You can also relate the scale index of the paper to the density scale of the negative, according to the chart.

KODAK PHOTOGRAPHIC PAPERS (TRAY PROCESSING)

The Kodak photographic papers discussed in this section are suited for scientific, medical, and industrial photography; all are processed by the customary develop-stop-fix-wash (tray processing) method familiar to photographers and darkroom technicians; all papers are available with the smooth, glossy surface (called "F" surface) that gives the best image definition. This surface should also be used when reproductions for publications are needed.

Here is a brief description of these photographic papers:

KODABROMIDE Paper is a fast speed enlarging paper that has many uses in nearly all branches of photography. An exceptionally wide development latitude makes this paper a good choice for fast production of prints from a variety of negatives. The high speed of KODABROMIDE Paper is particularly useful in printing very dense electron microscope negatives. The single-weight, F surface (glossy) paper is recommended. It has an image tone that is neutral black.

KODAK POLYCONTRAST Paper is a variable-contrast enlarging paper with medium speed and warm-black image tone. This is available in single-weight, white, glossy F surface. Contrast is varied by the use of KODAK POLYCONTRAST Filters, available in a KODAK POLYCONTRAST Filter Kit (Model A). This consists of seven filters that vary the contrast from Grades 1 through 4 and are used in the image-forming beam. When the correct exposure for any one filter is known, exposure for any of the other filters is predictable. Another big advantage of the variable-contrast enlarging paper is that one box of paper meets all contrast requirements. Therefore, there is no need of little-used grade of paper going out-of-date. This simplifies the keeping of photographic paper.

KODAK POLYCONTRAST Rapid Paper is a fast, variable-contrast material similar to KODAK POLYCONTRAST Paper, but it has a slightly colder image tone and is 2½ times faster. The high speed of this material is valuable in printing dense negatives and for enlarging small negatives to high magnifications (situations quite common in electron micrography). Contrast is varied similar to KODAK POLYCONTRAST Paper by using the KODAK POLYCONTRAST Filters in the KODAK POLYCONTRAST Filter Kit (Model A). This paper has the same storage advantages and features as other KODAK POLYCONTRAST Papers.

There are a wide variety of different photographic papers that can be used for printing the electron micrograph negative. However, the photographic papers listed previously are the recommended papers for this use. More information about Kodak photographic papers can be found in Kodak Publication No. G-1, *KODAK B/W Photographic Papers,* available from your photographic dealer.

Exposure Determination

The most common methods of determining exposure in printing are on-easel photometry, scale index (reading highlight and shadow densities), making test-strip exposures, or using a KODAK Projection Print Scale. These methods are briefly explained here but are described in more detail in Kodak publications dealing with printing (see "Kodak Publications," page 48). In all of the following methods, the recommended development time stays constant and the exposure is varied.

On-Easel Photometry. An increasingly popular method for determining print-exposure time utilizes a sensitive electronic photometer placed directly on the enlarging easel. By using the enlarger as the light source—with the negative in place and at the desired degree of enlargement—the photometer takes into account the influence of the entire optical system. The advantages of this on-easel evaluation include the immediate availability of the information (as opposed to reading densities away from the enlarger) and the high accuracy of measurement.

In printing electron micrograph negatives, the photometer's probe usually reads a *middletone area.* This single reading gives the correct exposure time in seconds, provided that the photometer has been previously correlated with a similar subject for which the correct printing time has been found by making a good test print. However, once this initial test has been made, the photometer will continue to give an extremely high percentage of good results—much better results than the "guessing" method gives. This provides a higher rate of successful prints and less wasted material.

Density Readings. This method has been previously explained in regard to

Table 5 **KODAK Photographic Papers for Electron Micrographs**

KODAK Papers	Description	Tint	Texture	Symbol	Weight	Grades	Image Tone
KODABROMIDE	A general purpose enlarging paper widely used for press, commercial, and scientific prints.	White	Smooth	F	Single Weight	No. 1,2,3,4,5	Neutral-black
KODAK POLYCONTRAST	A variable-contrast enlarging paper that is useful in commercial, industrial, and general photography. The contrast is varied seven degrees of contrast by the use of the KODAK POLYCONTRAST Filters.	White	Smooth	F	Single Weight	Variable Contrast (Use of POLYCONTRAST Filters)	Warm-black
KODAK POLYCONTRAST Rapid	Same features as KODAK POLYCONTRAST Paper but twice the speed. An excellent paper for printing dense negatives.	White	Smooth	F	Single Weight	Variable Contrast (Use of POLYCONTRAST Filters)	Warm-black
KODAK EKTAMATIC SC	A variable-contrast enlarging paper primarily used with a stabilization process for quick prints (proofing, newswork, police, medical, etc). Can also be tray processed.	White	Smooth	F	Single Weight	Variable Contrast (Use of POLYCONTRAST Filters)	Warm-black Neutral-black (tray processed)
	All papers use an OC (light amber) KODAK Safelight Filter.						

Figure 39—An illustration of the KODAK POLYCONTRAST Filter Kit (Model A).

the selection of the proper grade of paper (see page 31). Briefly, the darkest area of the negative is read on a densitometer or similar density-reading apparatus. This gives the *highlight density*. Now the *shadow area* is read (this is the brightest area where detail is present). Subtract the first value from the second. This value is the density scale of the negative; this can then be matched with the photographic paper being used to give the proper grade of paper.

Test-Strip Method. Cut one sheet of enlarging paper into six strips. Expose and process one strip at a time. Try to place the strip on the enlarging easel so that it will receive exposure from both the important highlight areas and the important shadow areas of your negative. A good "average" exposure is 15 seconds with the lens set at *f*/8. If your first strip is too dark, decrease the exposure time. If it is too light, increase the exposure time. Using this approach, you should arrive at the correct exposure after using one or two strips, which is much more economical than using several sheets of enlarging paper.

Use of KODAK Projection Print Scale. Another method for determining the proper printing exposure is by using the KODAK Projection Print Scale, Kodak Publication No. R-26, available from photo dealers. After composing and focusing your picture, place a sheet of enlarging paper on the easel, as you would to make a normal enlargement. Place the Projection Print Scale with the numbers reading correctly on the enlarging paper so that it covers the most important part of the subject. Expose the paper for 1 minute at *f*/8 and process it. After processing, examine the print in normal room light. Find the segment that shows the best exposure. The number in that segment is the number of seconds you should expose your print.

These are only several methods of exposure determination that can be used. There are others that can be found in publications dealing with printing.

Exposing Your Print

With the room lights out and the safelight on, make a final check of focus and composition. Then, be sure the enlarger lens is set the same as it was for your

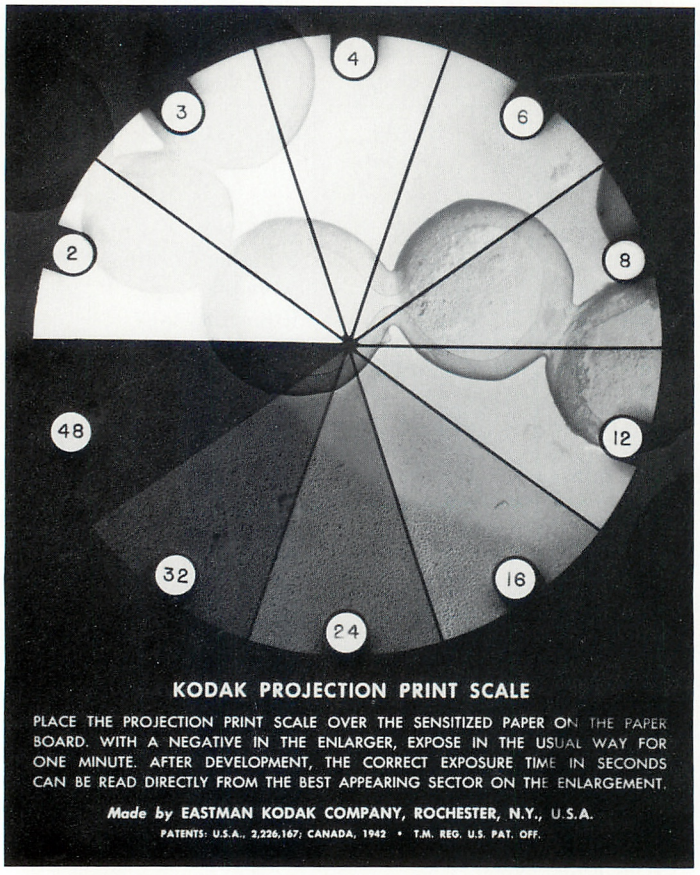


Figure 40—Use of the Projector Print Scale. Expose for 1 minute at *f*/8. Pick the best exposure and use the number of seconds in the circle.

best test print. If the enlarger has a point light source, expose with the lens wide open. However, neutral density filters may be needed to extend the exposure time. If you are using a condenser light source enlarger, stop down two *f*-stops. This will result in a flatter, sharper image. (Incidentally, stopping down a lens too far can cause a noticeable loss of definition.)

After setting the lens opening, remove the white focusing paper from the easel. This eliminates the possibility of "print-through" (halation and diffusion of the image) that may occur when a white background is used under single-weight enlarging paper during exposure.

You are ready to print your negative. Remove a sheet of enlarging paper from its lighttight envelope or box; put it in the easel, emulsion side (shiny side) facing the emulsion of the negative, and expose your print.

PROCESSING THE PHOTOGRAPHIC PRINT (TRAY-PROCESSING)

After the exposure has been made, the photographic paper must be processed. In the following table, you can find the recommended solutions, times, and temperatures.

Notes on Processing

Development. You can slide a print into the developer with the emulsion side either up or down. The developer should cover the entire print quickly, and no air bubbles should be trapped next to the emulsion. Constantly rock the tray with a gentle motion. All prints should be handled by the edges only.

Although you may be tempted, do not lift up the print during development. Doing so increases the possibility of print stains, particularly with partially exhausted developers. Also, if you hold a print close to a safelight during development, you may get safelight fog on the print. This will be evident as a gray appearance in the white borders. Any additional exposure may cause the highlights to go gray, resulting in a lowering of contrast and a loss of print quality.

Most Kodak enlarging papers should be handled and developed using a safelight equipped with a KODAK Safelight Filter OC (light amber) and a 15-watt bulb. The safelight should be at least 4 feet from the paper. Check the instruction sheet packaged with the paper for proper safelight recommendations.

This instruction sheet also gives time and temperature recommendations for processing the paper. As the print develops, it becomes darker. However, do not let the appearance of the print in the developer make you alter the development time. The best control of the print density (degree of darkness) is obtained by *altering the exposure time*, not the development time. Develop your test print for the recommended time and temperature that will indicate accurately the appearance of the finished print. If you *slightly* overexpose or underexpose the print, you can compensate *to a limited degree* by changing the development time. Developing for a longer time may result in a stained print. Developing for a shorter time may cause mottling or streaking.

There are other reasons for developing a print the recommended time rather than developing by "eye" or by the appearance in the developer. First, the safelight isn't adequate in brightness for judging print quality. Second, after the print has been in the fixing bath for a few seconds, the "veiling" effect of the unexposed and undeveloped portions of the emulsion clears. This considerably changes the appearance of the shadow areas.

Stop Bath. When development is completed, pick up the print by a corner, let it drain for a few seconds, and immerse it face up in KODAK Indicator Stop Bath solution. Leave the print in the solution 5 to 10 seconds, agitating it continuously. This solution stops the developing action and prepares the print for fixing. Do not keep the prints in the stop bath for a prolonged time. Doing so may cause "water soak," which appears as

Table 6 **Tray-Processing of Photographic Papers**

Step	KODAK Chemical	Time	Temperature	Comment
Development	DEKTOL (1:2) D-72 (1:2) EKTAFLO, Type 1 (1:9)	1½ minutes 1½ minutes 1½ minutes	20 C (68 F) 20 C (68 F) 20 C (68 F)	These are the recommended developers. Check for useful capacity.
Stop Bath	Indicator Stop Bath EKTAFLO Stop Bath Stop Bath SB-1	5-10 seconds 5-10 seconds 5-10 seconds	18-21 C (65-70 F) 18-21 C (65-70 F) 18-21 C (65-70 F)	These stop baths turn purple on exhaustion
Fixing Bath	Photo-Fix EKTAFLO Fixer Rapid Fixer (1:7)	6-10 minutes 6-10 minutes 3 minutes	18-21 C (65-70 F) 18-21 C (65-70 F) 18-21 C (65-70 F)	Use proper agitations and do not overfix
Wash or *Conserve Water*	Running water Clearing Bath after Fixer and rapid wash	60 minutes 2-3 minutes 5-10 minutes	18-23 C (65-75 F) 18-21 C (65-70 F) 18-23 C (65-75 F)	Separate prints to facilitate washing
Dry	Sponge excess water from prints, then use blotters or drying machine			

small translucent areas on the print. These areas may stain eventually or become noticeable if you tone the print.

Fixing. After the print has been in the stop bath for 5 to 10 seconds, pick it up, let it drain for a few seconds, and transfer it to the fixing bath or hypo. Place the print in the fixing bath face up. Agitate the print for a minute or two. Then rinse your hands in running water and dry them thoroughly. Put away your photographic paper. Now turn on the room light and examine the print. Quite possibly, you will be able to improve on the quality of your first print. The section entitled "Evaluating the First Print" on page 36 will help you judge print quality and decide what you can do to make a better print.

Finish fixing the prints for 5 to 10 minutes. If a large number of prints are being processed, a two-bath fixing method should be used. The prints are immersed for one-half of the fixing time (3 to 5 minutes) in each of two baths. After 200 8 x 10-inch prints have been fixed, the first bath is discarded and the second bath replaces the first. A fresh bath is mixed and now becomes the second bath. In this way, the first bath does most of the actual fixing while the second bath remains relatively fresh. This may be repeated five times then both baths should be discarded. This method will properly fix all prints.

Be sure to use the proper fixing time. Overfixing a print can cause it to fade. Also, the longer prints are fixed, the more difficult it is to wash the fixer out of the prints.

KODAK Hypo Clearing Agent. After the second fixing bath, rinse the print briefly in water and place it in a bath of KODAK Hypo Clearing Agent solution. Hypo Clearing Agent eliminates fixer and silver salts, and makes possible faster and more thorough washing. This means greater print stability with no adverse effect on the ferrotyping characteristics of glossy paper. The conservation of water is a big advantage of using this solution.

Use the Hypo Clearing Agent solution at 18 to 21 C (65 to 70 F). With agitation, treat single-weight or thinner papers for at least 2 minutes, double-weight for at least 3 minutes. Treatment times longer than 10 minutes should be avoided to prevent "water soak."

Washing. After treating the paper in Hypo Clearing Agent, wash single-weight or thinner papers for 10 minutes, double-weight papers for 20 minutes. The water temperature may be as low as 2 C (35 F). However, if you keep the temperature between 18 and 24 C (65 and 75 F), you will get greater print stability. Wash your prints in running water that flows fast enough to provide active agitation; however, do not let a stream of water fall directly onto the prints. You can use a KODAK Automatic Tray Siphon to convert any tray into a good print washer. Keep the prints separated, and turn them over often.

If you don't use Hypo Clearing Agent, wash the prints for 1 hour at 18 to 24 C (65 to 75 F). Improperly washed prints are subject to fading and yellowing with age.

To minimize print curl, bathe your prints in KODAK Print Flattening Solution after washing and before drying.

Drying. After you've washed your prints, place them on a clean, smooth surface, such as the bottom of a tray. To minimize drying marks, wipe the emulsion surface of each print with a viscose sponge. Sponging the print removes any sediment and facilitates faster drying.

Using a KODAK Photo Blotter Roll is one method of drying a small number of black-and-white prints. Place the prints face down on the blotter and roll it up. The corrugated cardboard backing of the blotter allows air circulation when the blotter is rolled up. When the prints are dry, they will be nearly flat, with a

desirable slight reverse-curl. Other methods of print drying include (1) placing the prints between blotter sheets, stacking prints and blotters alternately; (2) placing the prints face up on cheesecloth stretchers; and (3) using commercially available print dryers. The method used will depend upon the amount of prints that are produced and the type of surface needed. If the volume is high, and glossy surface prints are needed, then a commercial print dryer would be an excellent investment.

Figure 41—KODAK Automatic Tray Siphon—Converts any ordinary tray into an efficient print washer. Keeps the prints in motion and separated. Has faucet connector and 3 feet of ⅜-inch rubber tubing.

Ferrotyping. Ferrotyping, or glazing, is a means of imparting a high gloss to the surface of certain photographic papers. All Kodak F surface papers can be ferrotyped with this exception, KODAK POLYCONTRAST Rapid RC Paper. The RC indicates a resin non-fibrous coating on this paper. This new material absorbs very little moisture and, therefore, drys very rapidly. The surface of this paper has a medium gloss. Ferrotyping of F surface papers is accomplished by placing the emulsion surface in contact with a chromium-plated sheet, or by using a ferrotyping machine. The glossy surface increases the apparent tonal range of the print, and the absence of texture helps to reveal fine detail. Many publishers prefer this type of print surface for illustrations to be used for publication.

Composition

"Composition" means the arrangement of the lines and areas that make up your print. While entire books have been written on composition, there are a few basic pointers that will help you compose the most pleasing and interesting enlargement. It's usually best to have the center of interest neither right in the middle of the print nor too near the margins. Your eye should be drawn first to the area of interest.

Your print should tell its story as simply and clearly as possible. This means you should try to eliminate areas and details that are unnecessary or distracting. Often you can do this by shifting the enlarger easel, or increasing the enlargement.

Evaluating the First Print

You can inspect your first print under normal room light after it has been in the fixing bath a minute or two. An ideal "inspection board" is an inclined flat

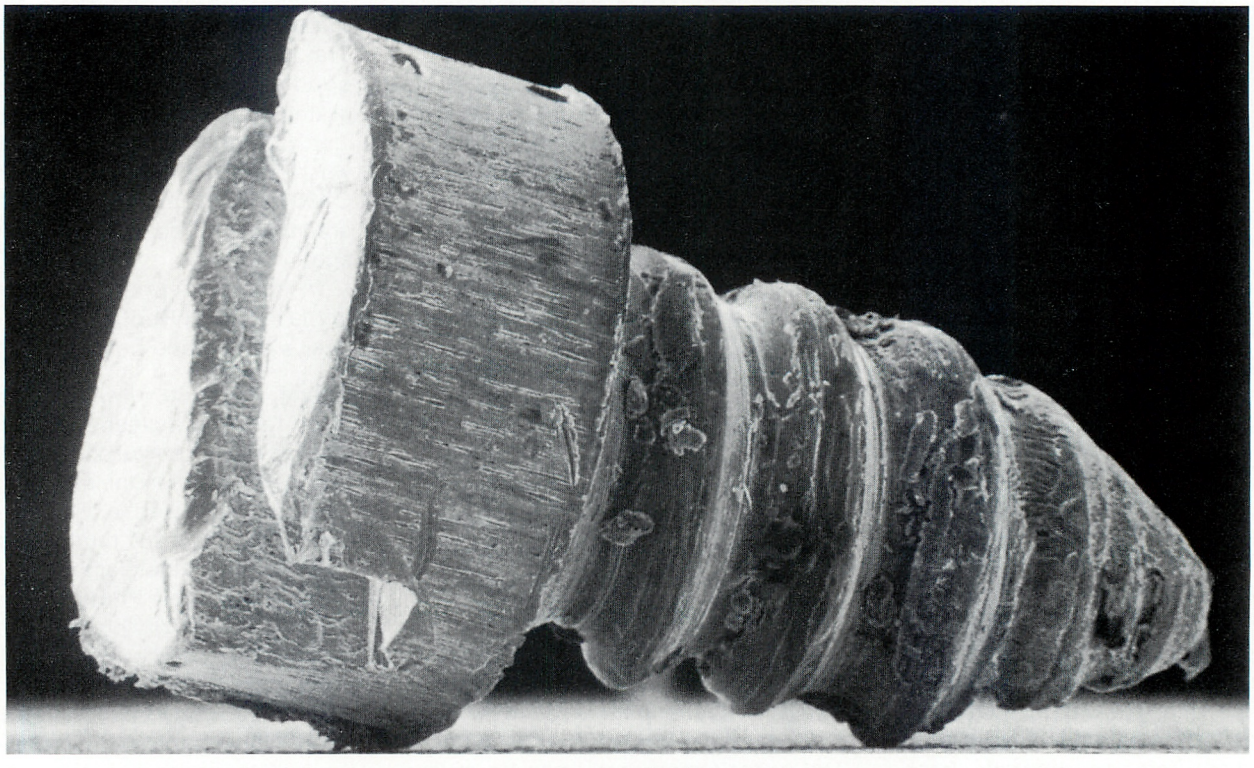


Figure 42—A jeweler's screw from the balance wheel of a watch. Magnification is 200×. This print illustrates proper exposure and printing to yield excellent detail and contrast.

surface, about 16 inches square, illuminated by a fairly bright light directly over the surface. However, a good substitute for an inspection board is the back of a large processing tray.

Rinse the print briefly in water and put it on your inspection board. Wipe the moisture from the print with a sponge or cloth. This is important, because a film of water on the print surface may give a false impression of print density.

How does your first print look to you? If an exposure series was made, the exposure should be right; the next thing to consider is contrast. For proper contrast, your print should usually have a white area in the highlight area, and a black area in the shadows. There should be a good range of grays in between these two extremes. To help decide on the best contrast for your print, you may want to print the negative on several grades of paper. Then compare the prints and decide which one reveals the most detail.

Exposure Time and Magnification

As you change the image size of your enlargement, a change in exposure time must be made. With some experience, you may be able to estimate the necessary increase or decrease. However, a more exact method is to use the Enlarging Computer in the KODAK *Master Darkroom* DATAGUIDE (Kodak Publication No. R-20) available from your photographic dealer. This computer is an aid in making correctly exposed enlargements when a new exposure time must be determined because of a change in magnification, lens opening, or POLYCONTRAST Filter.

Processing Several Prints at Once

Now that you have determined the contrast and exposure time, you may want to make several prints from this negative. If, for example, you want three or four prints, you will save time if you expose all of the prints first, and process them at the same time. In fact, unless it's hot or humid, you can expose all the prints first, and then process them later with good results. Several hours is the limit for storing exposed paper and maintaining good latent-image stability. Longer storage periods before development can cause a loss of image density.

When you process several prints at a time, handle them in the solutions much as you would shuffle a deck of cards. The prints should be emulsion side up in all solutions. This helps prevent air bubbles from being trapped at the emulsion surface, which keeps the solution from acting on the emulsion. Put the first print in the developer and follow it rapidly with the second, third, and fourth prints. Be sure that each print is covered by the solution. Then quickly take the bottom print and put it on top of the stack. Continue this rotation until all the prints are developed. If each print is immersed in the developer in rapid succession, the small differences in development time will be negligible.

Agitate the prints continuously in the stop bath, fixing bath, clearing agent, and wash water. This prevents unevenness in the prints caused by uneven action of the solutions.

Table 7 lists some of the more common problems and remedies that occur in print making.

Table 7 **Common Problems and Remedies in Print Making**

Problems	Probable Cause	Remedies
Yellow Stains	Insufficient agitation in fixer Exhausted fixer or stop bath	Agitate prints in fixer Mix fresh chemicals
Overall yellow stain in emulsion and paper base	Overdevelopment Stale, oxidized developer	Develop for recommended time Mix fresh developer
Mottled and unevenly developed image	Overexposure and underdevelopment	Reduce exposure and develop for recommended time
Blue spots or stains	Iron in wash water or solutions Chipped enamel trays	Install a water filter Dispose of chipped trays
Small black spots	Airborne chemical dust Chemicals in developer not properly dissolved	Mix powdered chemicals away from darkroom Dissolve chemicals completely when mixing developer
Gelatin on prints too soft Prints easily damaged Prints marked by cloth or blotters used for drying	Exhausted fixer Lack of hardener in fixer Wash water too warm Prints left too long in wash water	Mix fresh fixer Mix stop bath Wash prints as instructions indicate
Prints lack contrast	Dusty or fingermarked enlarger lens White light or flare in darkroom Wrong grade of paper Safelight fog	Clean enlarger lens Check darkroom for white light and flare Use higher contrast grade of paper Check safelight filters (cracks, bulb)

STABILIZATION PROCESSING

Stabilization is a method of processing black-and-white prints much more quickly than is possible by the customary tray-processing method described in the previous section. Stabilized prints are not permanent because the chemical reactions within the emulsion have been stopped only temporarily. However, such prints last long enough to serve a number of practical purposes. In fact, stabilized prints can remain unchanged for many months if they are not exposed to strong light, high temperature, or excessive humidity.

The main differences between stabilization processing and ordinary print processing are in the *speed of development* and in the method of treating the unexposed light-sensitive silver halide left in the emulsion after development. Stabilization processing is a machine operation. The prints are processed in 15 seconds and are slightly damp on leaving the machine. However, they dry completely in a few minutes at room temperature and are then ready for use. The emulsion of a stabilization paper, such as KODAK EKTAMATIC SC Paper, contains the developing agent; processing is achieved by applying an activator to the paper surface. The stabilizer is then applied to neutralize the activator and to convert any remaining silver halide to relatively stable, colorless compounds. These compounds remain in the paper. In conventional processing, the unexposed silver halide is dissolved by the fixer (hypo) and removed by subsequent washing. Thus, conventionally processed prints are stable for long periods. In stabilization processing, however, the silver halide is converted to *temporarily* stable compounds; therefore, the prints have a limited keeping time. Stabilized prints can, however, be made permanent by fixing and washing after their initial purpose has been served.

There are several types of stabilization processes, the monobath, a one-solution process, or a two- (or more) solution process. The monobath process is excellent for drawings and linework. However, the type most suitable for processing electron micrograph prints is the two-solution process that provides excellent-quality, continuous-tone prints.

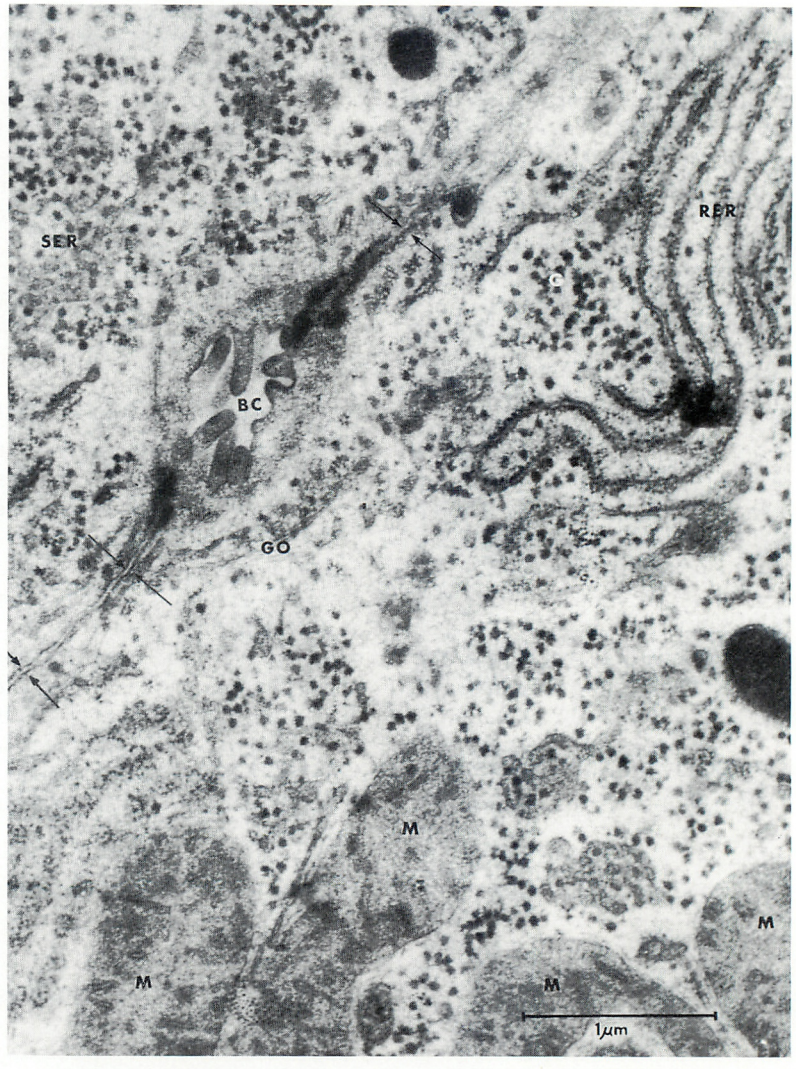


Figure 43—A TEM illustration where the main subject (BC) is not placed in the exact center of the print. This makes a more interesting print. Exposed on KODAK Electron Image Plate, printed on F-3 KODABROMIDE Paper (Magnification 25,000 ×).

The first solution is an activator; the second solution is a stabilizer.

KODAK EKTAMATIC Products for Making Stabilized Prints

The KODAK EKTAMATIC Products described here are for black-and-white print processing by the stabilization method. With these products, conventionally exposed prints can be processed and ready for use in a matter of seconds—prints of excellent quality that can be used for a great many purposes when permanence is not important. Moreover, the prints can be made permanent, if desired, by subsequent fixing and washing. No special skill or lengthy training is necessary to operate the KODAK EKTAMATIC Processor, Model 214-K. There are few, if any, photographic operations in which stabilization processing cannot be used to advantage when the savings in processing time and space are considered. The savings will repay the initial cost of the processor in a comparatively short time.

Use of Stabilized Prints. The uses for stabilized prints are almost as diverse as photography itself. This method is being used increasingly by photographers in the commercial, industrial, portrait, press, law-enforcement, and scientific fields.

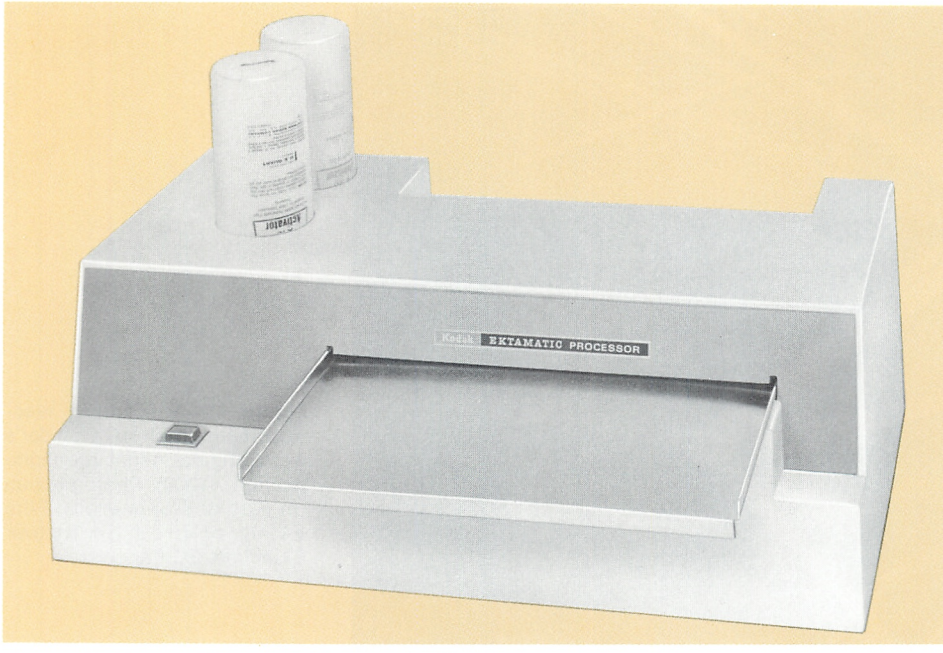


Figure 44—KODAK EKTAMATIC Processor, Model 214-K. A processed print is ready in 15 seconds.

When photography is used as a tool to support some other operation such as electron microscopy, the time lapse between receiving a negative and supplying prints has always been too long. The use of KODAK EKTAMATIC Products almost eliminates this waiting period. After the initial purpose of the print has been served, the prints can then be fixed, washed, and dried. They are then permanent and can be filed for future use.

KODAK EKTAMATIC SC Papers. The initials SC stand for selective contrast. This means that the contrast of the print can be varied from the equivalent of Grade 1 through Grade 4 in half-grade steps by using KODAK POLYCONTRAST Filters—the same filters described on page 32. This paper contains an optical brightening agent, which adds brilliance and contrast to the prints.

KODAK EKTAMATIC SC Papers are fast, projection-speed materials for processing with two-solution stabilization processors such as the KODAK EKTAMATIC Processor, Model 214-K.

This paper is available in F or A surface, glossy or smooth, single or light weight. When stabilization-processed the tone is warm black. However, the tone becomes neutral black if it is tray-processed in DEKTOL Developer.

KODAK EKTAMATIC SC Papers should be handled and developed under a KODAK Safelight Filter OC (light amber) used in a suitable safelight lamp with a 15-watt bulb at a distance of 4 feet. Three minutes under these conditions is considered to be the maximum safe time. KODAK variable-contrast papers depend upon exposure to blue and yellow light for contrast control; therefore, prolonged exposure to the safelight may cause a decrease in contrast.

KODAK EKTAMATIC SC Papers are supplied in standard sheet sizes, from 5 by 7 inches to 16 by 20 inches. Larger sheets and rolls can be supplied on special order.

Exposing KODAK EKTAMATIC SC Papers. These papers can be exposed with the type enlargers normally used by electron microscopists.

This enlarger should be either a point light source or a tungsten light source with a double condenser lens system. The lenses should be clean, the bellows in good condition, and the working area clean and free from clutter. To make an exposure on this paper, the same procedure that was used for making a conventional variable-contrast enlargement is used as described on page 33.

KODAK EKTAMATIC Processor. This is a two-solution stabilization processor of heavy-duty construction designed to withstand the wear and tear of daily use. The KODAK EKTAMATIC Processor, Model 214-K, accepts sheets and rolls of paper up to 14 inches wide. The smallest sheet that can be processed is 5 inches long.

An 8 by 10-inch print is processed and clear of the machine in 15 seconds. The paper is in a damp-dry condition, but dries completely in a few minutes in normal room atmosphere.

Solutions in the processor are replenished automatically by means of solution-level valves placed on the original plastic containers. Be careful to avoid contamination of the activator with the stabilizer—this will be indicated by chemical fog on the prints or, in severe cases, by a smell of ammonia. If this happens, remove the chemical tray and the processing-rack assembly from the machine, wash them thoroughly in water, and install fresh chemicals. For routine cleaning, refer to the instruction manual supplied with the processor.

Warning: Do not use aluminum foil under the KODAK EKTAMATIC Processor. If the activator solution is spilled on the aluminum foil, a chemical reaction results which can cause respiratory irritation. Plastic, wood, or cardboard can be substituted to protect the bench top.

KODAK EKTAMATIC Chemicals. Two solutions are required for processing KODAK EKTAMATIC SC Papers: KODAK EKTAMATIC A10 Activator and KODAK EKTAMATIC S30 Stabilizer. In this stabilization process, the developing agent is incorporated in the paper emulsion. Development is achieved by applying an alkaline activator to the emulsion surface. The stabilizer is then applied to neutralize the activator and to convert any remaining silver halide to *relatively* stable, colorless compounds. These solutions are supplied ready to use in plastic bottles of a size and shape that fit the processor. In stabilization processing, the temperature of solutions is not critical; 21 C (70 F) is ideal, but the useful range is 18 to 29 C (65 to 85 F).

Capacity of Solutions. Because stabilization processors vary in the amount

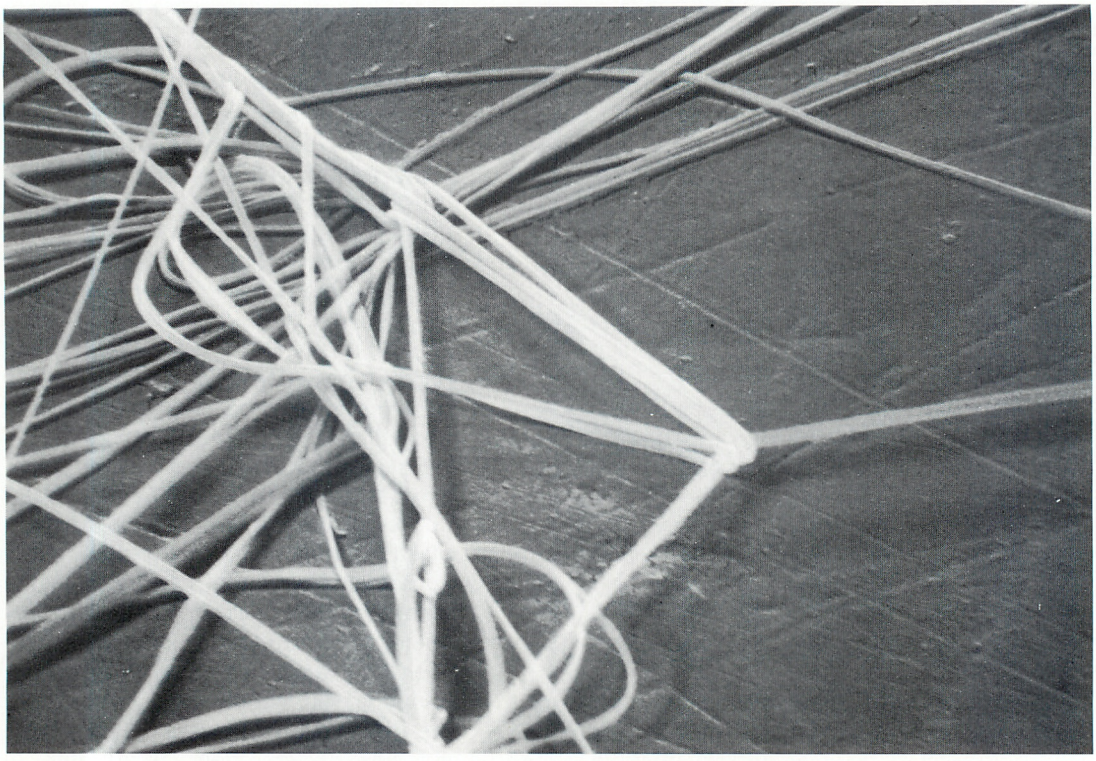


Figure 45—A scanning electron micrograph of a spider's web (original negative magnification X1000). Photographed on KODAK Commercial Film 4127, printed on KODAK Ektamatic SC Paper.

of solution needed to fill the trays, a valid solution capacity in square feet per gallon is not applicable. However, discard the solutions and clean the processor weekly or after about three hundred 8 by 10-inch prints (or the equivalent of other size papers) have been processed.

KODAK EKTAMATIC Chemicals will keep indefinitely in the original sealed packages, but storage below 5 C (41 F) or prolonged storage at temperatures in excess of 38 C (100 F) should be avoided.

Drying Stabilized Prints

Prints are damp on leaving the processor, but at room temperature will dry completely, without curl, within a few moments. *Accelerated drying by heat is not recommended*, nor can the prints be ferrotyped on hot drum machines unless they have been fixed and washed.

Keeping Stabilized Prints

The keeping time of stabilized prints is limited because the chemical reactions within the emulsion have been stopped only temporarily. Deterioration will eventually follow, especially after exposure to heat, light, and humidity. The time taken to produce a change in the condition of a print depends upon the degree and combination of these factors. Prints made on KODAK EKTAMATIC SC Papers will keep for many months if the storage conditions have been favorable, and if the chemicals were in good condition at the time of processing.

When permanent prints are required, the following after treatment is recommended: Fix the stabilized prints for 8 to 12 minutes in KODAK Fixer, KODAK Fixing Bath F-5 or F-6, or KODAK Rapid Fixer (1:7) at 18 to 21 C (65 to 70 F).

NOTE: KODAFIX Solution and KODAK EKTAFLO Fixer are not recommended for fixing stabilized prints.

Treat the prints for 2 minutes in KODAK Hypo Clearing Agent solution. Then wash, dry, or ferrotype them in the conventional way.

Tray Processing

KODAK EKTAMATIC SC Papers can be tray-processed in the same solutions used for normal photographic paper processing. KODAK DEKTOL Developer, diluted 1 part developer to 2 parts water, is recommended. The developing time is about 60 seconds at 20 C (68 F). The subsequent steps in processing, including drying or ferrotyping, are the same as for conventional papers.

Important Points in Stabilization Processing

Here are ten important points to follow in order to obtain high-quality results with stabilization processing:

1. Correct exposure is essential because development time is constant. Use an on-easel photometer to determine correct exposure.

2. Keep the processor clean. Follow the manufacturer's recommendations about cleaning and maintenance.

3. Use fresh chemicals. Observe the manufacturer's recommendations about the capacity and renewal of solutions.

4. Make sure that the processing trays in the machine are dry before adding the chemicals. Some stabilization solutions are not compatible with water.

5. Avoid contamination of the activator with stabilizer. This results in chemical fog on the prints.

6. Avoid handling unprocessed paper after handling stabilized prints. Stabilized prints are impregnated with chemicals that can easily stain unprocessed material.

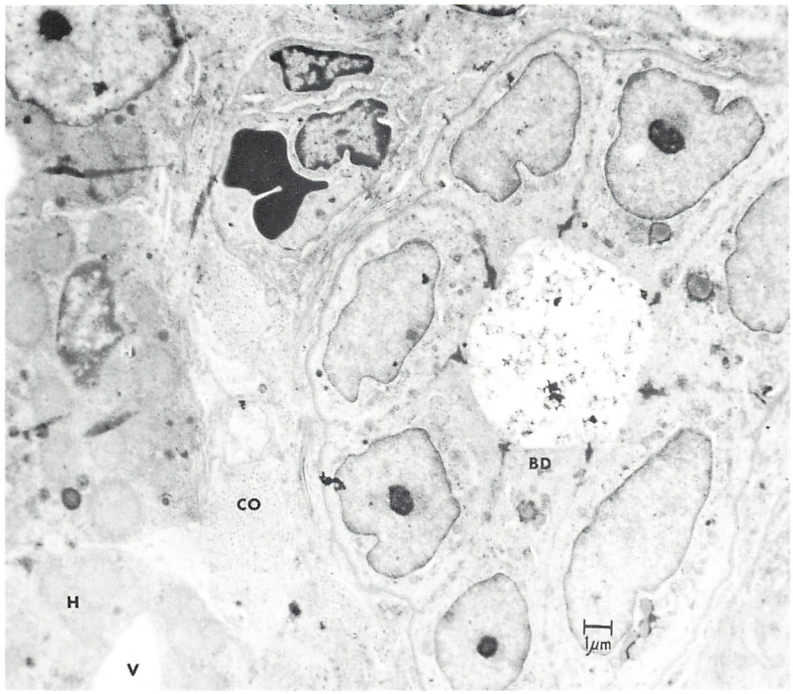


Figure 46—This electron micrograph was dodged to give an overall even print. Originally, it was too light near the magnification marker (3,500 ×).

7. Stabilized prints must not be washed unless they have been fixed in an ordinary fixing bath. Washing without fixing renders the prints sensitive to light.

8. Stabilized prints must not be heat-dried. The combination of heat and moisture stains the prints an overall yellowish brown.

9. Because stabilized prints are impregnated with chemicals, do not use the same racks or blotter for drying conventionally processed prints. For this reason, do not file stabilized prints in contact with negatives or any other valuable photographic records.

10. Make sure stabilized prints are dry before stacking or storing. The dry prints are stored at normal room humidity and at a low level of lighting. If long-term storage is required, it is best to fix and wash the stabilized print before any evidence of deterioration is apparent.

Mounting

Dry mounting of stabilized prints is not recommended as a normal procedure, but if necessary, it can be done in the following way, although there is risk of a change in color.

The print and any card used as an overlay must be quite dry. The temperature of the mounting press should not exceed 104 C (220 F), and the mounting time should be no longer than it takes for the paper to adhere to the mount (approximately 20 seconds).

CONTROL TECHNIQUES IN PRINTING

Up to this point, we have been concerned primarily with making "straight" enlargements—enlargements made without the use of any special control techniques. However, using control techniques, along with print-finishing techniques often makes the difference between a good print and an excellent one. Of course, not all negatives require special printing treatment. But since control techniques are quite useful, this section will cover some of the most important controls such as dodging, flashing, and combination printing. These are the tools you can use to produce high-quality enlargements.

Printing-In and Dodging

In many instances, the brightness range of a subject is beyond the range of tones that can be reproduced in a print. However, you can partially compensate for this in two ways: (1) You can give additional exposure to the highlight areas that would otherwise print too light. This is called "printing-in." (2) You can hold back light from areas that would otherwise print too dark. This is called "dodging."

If the print contains a light area that draws attention away from the main subject, or an area that would print too dark, with loss of detail, you can correct these by printing-in or dodging methods. Before exposing the paper, study the projected image and decide what treatment, if any, is needed. Dodging and printing-in should seldom be afterthoughts that occurred to you during the basic exposure. Time these operations so that they can be repeated accurately or changed in a future print. Guesswork here leads to a waste of time and paper. Use as little local control as is possible to get a good print. From force of habit, many printers tend to indulge in more of this work than is really necessary. If you find that many areas of the picture need dodging and burning-in, try a lower grade of paper. The paper grade being used may be too high in contrast for the negative.

Good workmanship demands that the effects of local control should not be obvious. For example, if you burn-in a highlight or a dense area of the negative, be sure that the exposure does not spill over into adjacent parts of the print. Otherwise, there will be a distracting dark ring around the burned-in highlight. Also, be sure to cover the highlight with exposure so that a dark patch does not appear in the center of it. In dodging or shading a shadow area, shade the affected area evenly so that the shadow does not become too light.

You can easily make your own printing-in and dodging tools from wire, black tape, and dark paper or cardboard. When using these tools, always keep them moving to eliminate any indication of these special control techniques on the finished print.

Printing-in. A piece of dark-colored card with a hole cut in its center is used for this operation. The size of the hole depends both on the size of the area to be burned-in and the lens aperture. To get a soft edge, when the lens is stopped down to a small aperture, use a small

hole and hold the card nearer the lens during the burning-in exposure. In treating large highlights or dense areas, cut a slightly ragged hole in the card. This provides a soft outline and enables you to hold the card nearer the easel for better control. To burn-in a very small highlight, just push a pencil through a piece of thin card and hold the card close to the easel.

Skilled printers often form a hole with their two hands for burning-in prints up to 8 × 10 inches or even larger. This method has an advantage in that the hole can be instantly varied in both shape and size to suit the particular area of the print. However, the using of this technique takes some practice, but is very flexible and time-saving. When burning-in by any method, keep the card or the hands moving throughout the exposure; otherwise, the print will not have a natural appearance.

Dodging. Again, you can use your hands for shading parts of a negative that may print too dark. With large prints, however, you will probably need to use a piece of card. In some cases, the card should be cut to a shape that conforms to the area you are shading. If the area to be dodged or shaded is in the center of the picture, use a paddle-type dodger. This is a circular piece of card attached to the end of a thin wire about 10 inches long. During the exposure, always keep the wire moving from side to side, to avoid a light bar on the print caused by the shadow of the wire. A paddle is particularly useful for reducing the exposure on areas that print too dark. Keep a set of these paddles handy. The most useful sizes range from ½ inch to 2½ inches in diameter. Whatever method you use for shading, keep the dodger moving throughout the exposure.

Flashing

"Flashing" is intentional fogging of the paper in a controlled manner. Use this technique when you want to reduce the contrast of a section of your print and when you want to subdue near-specular highlights.

There are two ways you can flash a print. One way is to use a small pen-type flashlight. Attach a cylinder of black paper over the lamp end of the flashlight so that you can flash small areas without affecting the remainder of the print. You can do the flashing during the normal print exposure if the flashlight projects a sufficiently fine beam of light (make sure you don't hold the flashlight in the enlarger light beam). Or, you can do the flashing after the normal exposure. In this case, place a red filter over the enlarger lens before you flash the print. The projected image is used as a guide without adding to the general print exposure.

The second technique of flashing involves using the enlarger as a light source. After you've made the normal print exposure, remove the negative from the enlarger. Stop the enlarger lens down to its smallest opening. Then flash the print where necessary, using your ordinary printing-in tools such as a piece of cardboard with a hole cut in it. The disadvantage of this technique is that you do not have the projected image to serve as a guide.

It's not a good policy to flash a print when it's in the developer. Since the image tone of a print often changes as it develops, the area of a print that is flashed during development may have a tone different from that of the remainder of the print.

Don't overflash—better too little flash than too much.

Combination Printing

"Combination printing" is probably the most rewarding (and demanding) control technique. In combination printing, two (or more) negatives are used to make one print. In electron micrographs, the main goal is to combine two or three negatives to illustrate the subject material being discussed. The combination will usually consist of a low-magnification micrograph of the subject plus a higher magnification showing greater detail of a specific area. These can be combined in several ways. Here is one method to use:

1. Determine the correct exposure for each negative by making a test exposure, with the enlarger set at the magnification you're going to use. Record the enlarger position (magnification) and the correct exposure for each negative.

2. Expose the low-magnification area of the print, and at the same time, use a card (cut to the contour of the higher magnification) to hold back exposure to the high-magnification area. This card can be circular, square, or whatever shape that will make the best combination print. In this case, the card is held steady a few inches above the easel.

3. Replace the first negative with the high-magnification negative. Print this negative, and at the same time use a second card to prevent any light from getting to the previously exposed area. This card is cut to the contour of the low-magnification area—use the other half of the card you used in step 2.

The results should be a well-balanced combination print from the two negatives. This method has the added advantage of lowering the number of illustrations needed, and therefore, lowering the publication cost. A good illustration like this is well worth the extra time and effort it takes to make it.

Another method of making a composite print is this: make separate prints at the different magnifications, cut them apart, and reassemble a composite print. If this is done carefully, it will be just as effective, as the previous method.

Specialized Printing (Printing Scratched Negatives)

In electron microscopy, there are times when an important negative may get scratched. If the negative is printed in the usual method, the surface scratches in the negative will print as white lines that are very obvious and extremely difficult to retouch. One solution to this problem is to fill in the scratches with *immersion oil* (the same oil used in regular photomicrography). This oil has a refractive index of 1.5 that is similar to the gelatin in the film and to the film base. Therefore, because the gelatin, film base, and immersion oil all have similar refractive indices, internal reflections caused by the scratches are subdued when the negative is printed.

Here is a description of the method that can be used in printing scratched negatives:

1. Some negative holders include a glass plate on top and bottom for holding the negative flat. If this type of holder is not available, use two thin pieces of glass slightly larger than the negative. Hinge one side of these glass plates with water-proof tape. Clean all surfaces and open the glass plates.

Figure 47—A scanning electron micrograph of the head of an ant *(cryttocerus texanus santachi)* at 75× magnification. This was photographed on KODAK Commercial Film 4127 and printed on KODABROMIDE F-2 Paper.

Figure 48—In mounting prints where directional lighting is evident, the light should appear to come from the top of the print with the shadows pointing down. This is a scanning electron micrograph of a diamond-studded dental drill (75×).

2. Apply a small amount of immersion oil in the center of each glass plate and the negative, close the plates, and tape them securely. Apply pressure to remove any air bubbles.

3. Remove any excess oil that has been squeezed out of this holder by using a cloth dampened with alcohol.

4. Print the negative in the normal way.

5. Remove the negative from the holder, immerse the film in alcohol, wash, and dry in the normal way.

6. Remove any remaining oil from the holder, enlarger, etc, with a cloth saturated with alcohol.

The immersion oils used in this method are Crown Immersion, Shillaber's A, or Cedarwood. These are available from scientific supply firms.

Print-Finishing Techniques

After you've processed your print, there are a number of finishing techniques you can use to improve your print.

These techniques include mounting—attaching your print to a proper board; spotting—filling in any white spots on the print with dyes; etching—removing dark spots from the print and, finally; titling—complete identification of the print. This section briefly describes these techniques.

Mounting. A mount separates the print from its surroundings, and therefore emphasizes the print. Usually a special mounting material, called mounting board, is used for this purpose. A well-chosen mounting board directs attention to the print, and not to itself. Mounting boards used with electron micrographs protect and display your finished work.

To attach a print to a mounting board, most photographers use an adhesive material such as KODAK Dry Mounting Tissue. This is a heat-sensitive tissue that is applied to the back of the print. The print and tissue are trimmed together, positioned upon the mounting board, and then placed into a dry-mounting press where heat and pressure are applied. They may also be mounted with a household iron. The temperature of the press for black-and-white prints is 93 C (200 F) for one minute.

Another way of mounting prints is to use overlay mounts available from art- or photo-supply stores. There is no actual mounting involved. The print is slipped between the overlay and the mounting board. Overlay mounts are fine for display or temporary use.

Spotting. Despite all the precautions you take against dust and dirt, most prints end up with at least a few white spots caused by dirt on the negative in the enlarger. You can fill in the spots by using a spotting brush and dyes. The brushes (such as KODAK Deluxe Spotting and Coloring Brushes) are available in several sizes. A No. 0 or No. 2 brush is good for general spotting. For spotting black-and-white prints, you can use KODAK Spotting Colors, which are dry dyes supplied in a set of three colors: black, white, and sepia. Another popular type of spotting dye is a liquid dye called Spotone.

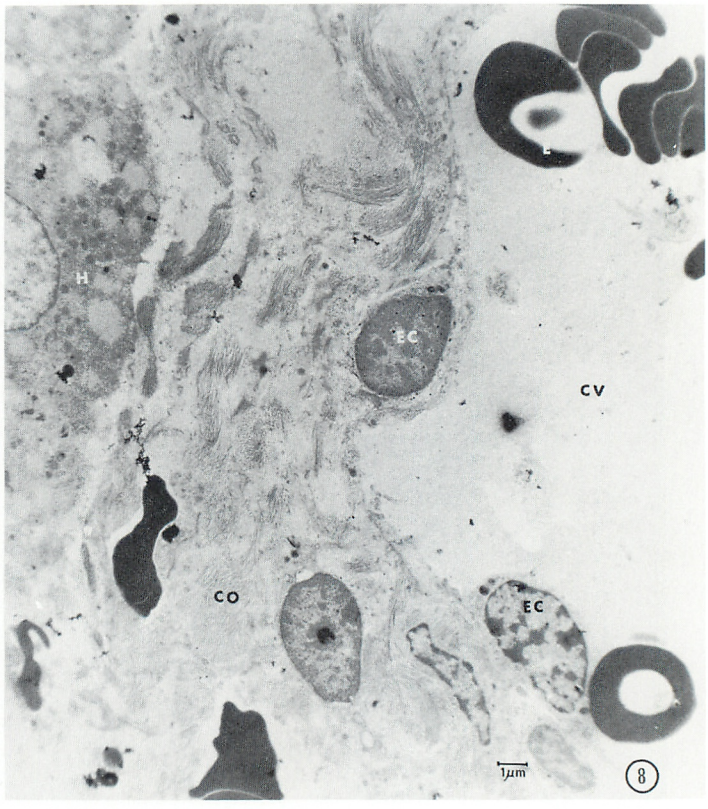


Figure 49—A complete illustration for a report or journal is numbered, includes a magnification scale, and identifies the main parts of the illustration.

To spot your print, dampen the spotting brush slightly and pick up a little spotting dye of the right color on the tip of your brush (you can mix colors if necessary). Rotate the brush to make a fine point. Apply the dye with a dotting or stippling motion until the dye matches the tone of the surrounding area—and the white spot will disappear. It's better to begin with the dark areas and work toward the lighter areas as the dye works out of the brush. If the dye bubbles when you apply it, the brush is too wet.

Etching. Sometimes your prints may have some small black spots caused by dirt on the film before it was exposed. You can eliminate these spots by etching them with a good-grade surgical steel knife, such as a KODAK Etching Knife.

The first time you do any print etching, practice on a scrap print. Scrape the emulsion very slightly with the knife—don't dig at it. Unlike spotting, which should be done after ferrotyping, etching is done *before* ferrotyping because etching disturbs the print surface.

Titling. Printing the title of your micrograph and your name just below the print adds the finishing touch. Keep the title short and simple. Ideally, the first letter of the title should start even with the left edge of the print. Print your name in the lower right corner in letters to match the title. The last letter of your name should be flush with the right edge of the print. Now add stick-on letters, a magnification scale, and any other identification that will complete the print.

Prints for Reproduction. In making prints for reproduction or for copying, avoid high contrast and shadows or highlights without detail. The prints should be a little softer than those made simply for viewing. Good coverage or detail in the highlights is perhaps the most important point here. If you make several prints for reproduction which will be printed in a publication, be sure they are similar in density and contrast. It is a good idea to consult the printer or the engraver to find out the kind of prints they prefer to work with, or the print quality that best suits the method of reproduction being used.

Prints for reproduction or copying should be made on a coldtone, smooth-surface paper such as an F surface or an N surface Kodak paper. The N surface is more suitable when much airbrush work or retouching is required. Again, however, the publisher's preference should be considered.

More information on printing, mounting, etc, can be found in Kodak Publication No. AG-16, *Enlarging in Black-and-White and Color,* and No. G-5, *Professional Printing in Black-and-White*—publications available from your photo dealer.

COPYING ELECTRON MICROGRAPHS

Many occasions arise when electron micrographs are needed to illustrate and to visually complement scientific, medical, or industrial presentations. An easy method of copying electron micrograph negatives onto 35mm format for making 2 x 2-inch transparencies for projection will be discussed.

The equipment necessary is a camera, a camera support, and a source of transmitted illumination. The ideal camera is a single-lens reflex camera with interchangeable lenses and through-the-lens metering system. By the use of supplementary lenses or a bellows unit, additional enlargement of any part of the negative can be accomplished. The camera support can be an unused enlarger stand, a commercial copy stand, or even a tripod. The illumination can be provided by any uniform commercial illuminator, such as a dental x-ray illuminator.

The method used is similar to standard copying techniques. The electron micrograph negative, emulsion side up, is placed on the illuminator. A dark mask with an opening only for the area to be

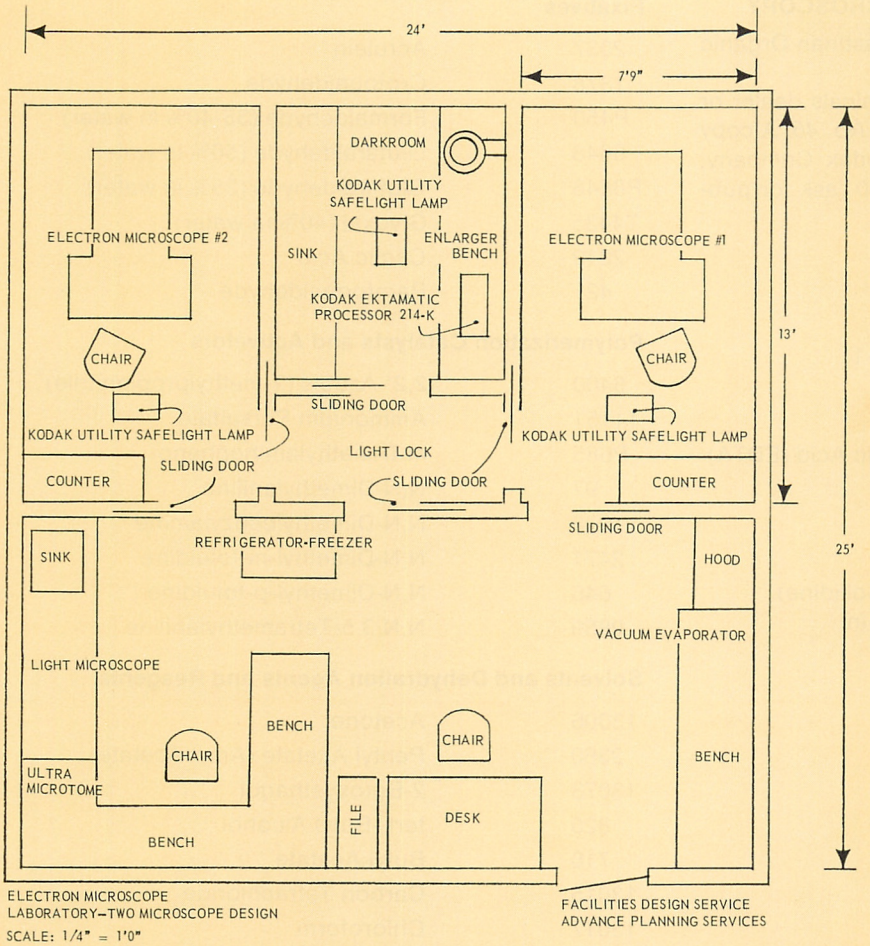


Figure 50—A basic electron microscope laboratory design.

photographed is used to eliminate any glare and surrounding stray light. The camera is attached to the copying stand with focusing accomplished by moving the whole camera up and down. All vibrations must be eliminated (use cable release on camera, shock mount the copy stand, etc).

The black-and-white films will give positive images similar to the paper prints made from these negatives. There are two films recommended. The first is KODAK High Contrast Copy Film 5069 readily available in 20-exposure rolls. This film has very slow speed, but high resolving power and extremely fine grain which will reproduce the electron micrograph negative detail. Its contrast can be adjusted by development. When copying low and medium contrast electron micrograph negatives, use KODAK DEKTOL Developer (1:1) for 6 minutes at 20 C (68 F). For high contrast negatives, use a lower contrast developer such as KODAK Developer D-76 for 4 minutes at 20 C (68 F).

The second suggested copying film is EASTMAN Fine Grain Release Positive Film 5302, the film recommended for 35mm photography in electron micrography. This film is extremely fine-grain, with high resolving power. Exposure and development conditions control the final slide. When copying low and normal contrast electron micrograph negatives, use a high contrast developer such as KODAK DEKTOL Developer (1:1) for 2 minutes at 20 C (68 F). For high contrast negatives, use a low contrast developer such as KODAK Developer D-76 for 2 minutes at 20 C (68 F).

The processed slides are mounted in either glass or cardboard mounts, properly identified, and projected. An advantage of this copying method is the efficient storage of these slides over the larger size original glass plates or films. More information on copying and slide making can be found in the Kodak publications listed in the Reference Section.

BASIC ELECTRON MICROSCOPE LABORATORY DESIGN

To help the electron microscopist design his laboratory, we are including a basic two-instrument design. This design includes an extensive preparation room and a complete darkroom. Only the fundamental equipment has been presented here, with ample space left for the addition of specialized equipment later on. The electron microscopes are placed adjacent to the darkroom so that the loading and unloading of film and plates is convenient. The power supplies for the electron microscopes are located in a service area some distance away from the microscopes to prevent stray magnetic fields from affecting the stabilization of the electron microscopes. A water supply (if necessary) is located near the instrument to cool the oil diffusion pumps. However, a small refrigeration unit that cools and recirculates the water through the instruments may be ideal for this use.

The electron microscope rooms are equipped with normal tungsten work lights and safelights. The tungsten lights are used when servicing the instruments and when training personnel, while the safelights are used when viewing and photographing samples and when entering the darkroom. The lighttight sliding doors in this design can be changed to meet the needs of the laboratory.

This design will need to be modified and enlarged if more than two electron microscopes are being installed. In the initial planning stage, ample room should be left to add another microscope at a later date. With the many applications of the transmission electron microscope and the added information obtained from the scanning electron microscope, a one- or two-microscope laboratory should plan for future expansion.

More information about design of laboratory and darkrooms is available by writing to electron microscope manufacturers who will supply layout and installation requirements for their instruments. For more information about other photographic laboratories and darkrooms, write to Eastman Kodak Company, Facilities Design Service, Professional and Finishing Markets Division, 343 State Street, Rochester, New York 14650.

EASTMAN CHEMICALS FOR ELECTRON MICROSCOPY

As a convenience to electron microscopists, Eastman Organic Chemicals* offers the following products.

For sizes and prices, see your organic chemicals dealer or refer to *EASTMAN Organic Chemicals Catalog No. 46.* A copy of this catalog is available from Eastman Kodak Company, Department 412-L, Rochester, New York 14650 (ask for publication No. JJ-1).

Eastman Organic Chemical Cat. No.	Description
Buffers	
4833	2-Amino-2 (hydroxymethyl)-1, 3-propanediol (TRIS)
7427	3,3-Dimethylglutaric Acid
5416	(Ethylenedinitrilo) tetraacetic Acid (EDTA)
P6274	4-Ethyl Morpholine
690	Maleic Acid
227	Sodium Acetate
X4815	2,4,6-Trimethylpyridine (s-Collidine). Specially purified for use in electron microscopy.
Contrast Media (Staining)	
1975	Lead Acetate
1460	Phosphotungstic Acid
491	Silver Nitrate
1973	Sodium Tungstate
10869	Uranyl Formate
10391	Vanadyl Sulfate
Embedding Media and Reagents for Polymer Modification	
5521	Acrylamide
P5161	Acrylonitrile
P9353	2-(Diethylamino) ethyl Methacrylate
P7073	2-(Dimethylamino) ethyl Methacrylate
507	Epichlorohydrin
4789	1,2-Epoxyethylbenzene
1099	Gelatin (Purified, Calfskin)
5247	Gelatin (Purified, Pigskin)
P8366	2-Hydroxyethyl Methacrylate
P8370	Hydroxypropyl methacrylate
5703	Methacrylic Acid
8334	Methyl Methacrylate
2068	Propylene Oxide
1465	Styrene
Fixatives	
2037	Acrolein
1878	Crotonaldehyde
P450	Formaldehyde (35-40% in water)
8648	Glutaraldehyde (50% in water)
P8648	Glutaraldehyde (25% in water)
T4435	Glyoxal (40% in water)
2219	Osmic Acid
421	Paraformaldehyde
Polymerization Catalysts and Activators	
6400	2,2′-Azobis (2-methylpropionitrile)
11151	Ammonium Persulfate
P685	m-Dimethylaminophenol
97	N,N-Dimethylaniline
1973	N,N-Dimethylbenzylamine
2471	N,N-Dimethyl-m-toluidine
646	N,N-Dimethyl-p-toluidine
9859	N,N,3,5-Tetramethylaniline
Solvents and Dehydration Agents and Reagents	
13006	Acetone
2360	Pentyl Acetate (Amyl Acetate)
13073	2-Butoxyethanol
820	tert.-Butyl Alcohol
710	Butyl Acetate
13015	Carbon Tetrachloride
13017	Chloroform
13020	1,2-Dichloroethane
13024	p-Dioxane
13031	Isopropyl Alcohol
13032	Methanol
520	Methyl Acetate
13034	Methyl Sulfoxide
2068	Propylene Oxide
9323	Sulfolane
13037	Toluene
302	Benzoin (UV stabilizer for polymers)
C8687	Cresyl Violet (RNA stain)
1403	Dibutyl Phthalate (plasticizer)
2010	Mercaptobenzanilide
8474	2-Methoxy-2phenylacetophenone (Benzoin Methyl Ether) (UV stabilizer for polymers)
7372	Thiocarbohydrazide (visualization agent for osmic acid fixation)
7386	Trifluoroacetic Anhydride (derivative forming agent before osmic acid fixation)

Other solvents, dehydration agents, and reagents can be found in the *EASTMAN Organic Chemicals Catalog No. 46,* in the sections on ACS reagent acids, bases, and solvents; solvent for chromatography; and dyes and biological stains.

*Unlike photographic products, which are distributed through photo dealers, EASTMAN Organic Chemicals are available through laboratory supply houses or on direct order ($25 minimum in the United States and Canada; $50 overseas) from Eastman Kodak Company, Eastman Organic Chemicals, Rochester, N.Y. 14650. They are neither intended nor sold for household use. Eastman Organic Chemical Catalog Number and Description should be given in the order.

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BOOKS OF INTEREST

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Barer, R., and Cosslett, V. E., *Advance in Optical and Electron Microscopy.* Academic Press; Volume 1, 1966; Volume 2, 1968; Volume 3, 1969; Volume 4, 1971. Recent advances in electron microscopy are explained in each volume.

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Heidenreich, R. D., *Fundamentals of Transmission Electron Microscopy.* New York, Interscience Publishers, Inc., 1964. This book deals with theory of the transmission electron microscope.

James, T. H. and Higgins, G. C., *Fundamentals of Photographic Theory, Second Edition.* New York, Morgan and Morgan, 1960.

Kay, D., *Techniques for Electron Microscopy.* Oxford, England, Blackwell Scientific Publications Ltd., 1965. A reference book of preparation methods for biological and nonbiological specimens.

Mees, C. K., and James, T. H., *The Theory of the Photographic Process, Third Edition.* New York, MacMillan Company, 1966.

Mercer, E. H., *Electron Microscopy—A Handbook for Biologists.* Oxford, England, Blackwell Scientific Publications Ltd., 1961.

Pease, D. C., *Histological Techniques for Electron Microscopy.* Academic Press, Inc., 1964. This book deals with preparation of biological specimens for the electron microscopist.

Siegel, B. M., *Modern Developments in Electron Microscopy.* Academic Press, 1964. This book contains chapters on physics of the electron microscope, ultramicrotomy, thin metal samples, histology, bacteria, and biological macromolecules.

Slayton, E. M., *Optical Methods of Biology.* Wiley-Interscience, 1970.

Swift, J. A., *Electron Microscopes.* New York, Barnes and Noble; London, Kogan Page, 1970. This book has collected information about various types of electron microscopes and attachments available.

Thomas, G., *Transmission Electron Microscopy of Metals.* John Wiley and Sons, Inc., 1962. This book deals with preparation of metallurgical specimens for transmission electron microscopy.

Thorton, P. R., *Scanning Electron Microscopy.* London, Chapman and Hall, Ltd., 1968. This book deals with the theory of the scanning electron microscope. Applications of the instrument are discussed.

Zworykin, V. K., *Electron Optics and the Electron Microscope.* New York, John Wiley and Sons, 1945.

In the field of electron microscopy, many publications can become outdated rapidly. Techniques that are used today are replaced by simpler methods tomorrow. To keep up to date with new methods and applications that are being used, the microscopist should attend scientific meetings and symposiums. Papers that are presented at these meetings are usually available in published form (proceedings). Here is a list of the proceedings of recent electron microscope conferences.

Uyeda, R., *Proceedings of the 6th International Conference on Electron Microscopy.* Tokyo, Maruzen Company, 1966.

Titlbach, M., *Proceedings of the 3rd European Regional Conference on Electron Microscopy.* Prague, Publishing House of Czechoslavak Academy of Sciences, 1964.

Bocciarelli, D. S., *Proceedings of the 5th European Regional Conference on Electron Microscopy.* Rome, Tipagrafia Poliglotta Vaticana, 1968.

Proceedings of the 25th (1967), 26th (1968)—(to the present year) Anniversary meeting of the Electron Microscopy Society of America. Copies of these proceedings (the papers that were given at these meetings) are available from C. J. Arcenaux, Claitor's Publishing Division, 3165 Acadian Street, Baton Rouge, Louisiana 70821.

Proceedings of the 1st (1968), 2nd (1969)—(to the present year) Symposium on Scanning Electron Microscopy. Copies of these proceedings are available from SEM Symposium office, IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616.

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The following list is for information only. Inclusion in this list does not guarantee endorsement by Eastman Kodak Company, but only acknowledges the availability of the products. The lists are presented as a convenience to those persons using this publication. A more complete list can be found in "Guide to Scientific Instruments" published by the American Association for the Advancement of Science, 1515 Massachusetts Avenue, Washington, D.C. 20005.

Electron Microscope Manufacturers

AEI Scientific Apparatus, Inc., 500 Executive Blvd., Elmford, NY 10523

AMR Corp., 149 Middlesex Turnpike, Burlington, MA 01803

Applied Research Labs, P.O. Box 129, Sunland, CA 91040

Balzer High Vacuum Corp., P.O. Box 10816, Santa Ana, CA 92711

Cameca Instrument, Inc., 101 Executive Blvd., Elmford, NY 10523

Coates and Welter Ins., 777 N. Pastoria Ave., Sunnyvale, CA 94086

Elektros, Inc., 10500 S.W. Cascade Dr., Tigard, OR 97223

ETEC Corp., 3392 Investment Blvd., Hayward, CA 94545

ISI International Sci. Ins. Inc., 970 San Antonio Rd., Palo Alto, CA 94303

JEOL USA, Inc., 477 Riverside Ave., Medford, MA 02155

Kent Cambridge Scientific, Inc., 8020 Austin Ave., Morton Grove, IL 60053

Perkin-Elmer Corp., P.O. Box 10920, Palo Alto, CA 94303

Philips Electronic Inst., 750 S. Fulton Ave., Mount Vernon, NY 10550

Siemens Corp., 186 Wood Ave., Iselin, NJ 08830

Ultrascan, Inc., 18530 S. Miles Pkwy., Cleveland, OH 44128

Carl Zeiss, Inc., 444 Fifth Ave., New York, NY 10018

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E. Leitz, P. O. Box 129, Rockleigh, NJ 07647

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Enlargers

BEEM, P. O. Box 132, Jerome Avenue Station, Bronx, NY 10468

Durst, Inc. (USA), Ehrenreich Photo-Optical Ind., Inc. 623 Steward Avenue, Garden City, NY 11530

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Bessler Photo Marketing Co., 219 South 18th Street, East Orange, NJ 07018

Glass Slides and Coverslips

Corning Glass Works, Lab Products Dept., Corning, NY 14830

Arthur H. Thomas, Inc., P. O. Box 779, Philadelphia, PA 19105

VWR Scientific, P. O. Box 3200, San Francisco, CA 94119

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LKB Instrument, Inc. 12221 Parklawn Drive, Rockville, MD 20852

Polysciences, Inc., Paul Valley Industrial Park, Warrington, PA 18976

Ted Pella Company, P. O. Box 510, Tustin, CA 92680

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Pako Corporation, 6300 Olson Highway, Minneapolis, MN 55440

Ultramicrotomes

American Optical Corp., Instrument Div., Sugar & Eggert Rds., Buffalo, NY 14215

E. Leitz, Inc., Link Drive, Rockleigh, NJ 07647

LKB Instrument, Inc., 12221 Parklawn Drive, Rockville, MD 20852

Ivan Sorvall, Inc., Pearl Street, Norwalk, CT 06856

Vacuum Equipment

Cole-Parmer Instrument Co., 7425 North Oak Park Avenue, Chicago, IL 60648

Denton Vacuum, Cherry Hill Ind. Ctr., Cherry Hill, NJ 08034

Edwards High Vacuum, Inc., 3279 Grand Island Blvd., Grand Island, NY 44072

Film-Vac, Inc. 149 South Dean Street, Englewood, NJ 07631

VWR Scientific, P. O. Box 3200, San Francisco, CA 94119

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Eastman Kodak Company supplies more than 750 publications that provide authoritative, up-to-date technical information about Kodak products and their applications in many fields. These publications are listed in the *Index to Kodak Information,* Kodak Publication No. L-5. You can obtain an *Index* by writing to Department 412-L, Eastman Kodak Company, Rochester, New York 14650.

Here are some of the items in the *Index* that are of particular interest to scientific photographers:

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Kodak Materials for Electron Micrography (P-204) 10

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Gaseous-Burst Agitation in Processing (E-57) 20

How Safe Is Your Safelight? (K-4) 10

Most of these publications are available through your photo dealer. In some instances, however, publications dealing with specialized photography are not stocked by all dealers. Any of the items may be ordered directly from:

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Figure 51—Scanning electron micrograph of a bee stinger through the "eye" of a needle (150 ×).

Table 8 **Common Faults In Electron Microscopy**

Appearance of Negative	Possible Causes	Remedies
1. Low contrast	Incorrect sample preparation	Use stains or shadowing to enhance contrast.
	Objective aperture not used	Use proper aperture.
	Improper exposure	Increase exposure.
	Improper development	Develop for higher contrast.
2. Hazy, unsharp image, lack of detail	Sample or thermal drift	Prepare sample correctly.
	Instrument instability	Check instruction manual.
	Contaminated column	Clean column.
	Improper processing	Use recommended processing procedure.
3. Slight line around specimen Asymetrical diffraction line	Incorrect focus Astigmatism in objective lens	Use proper focus Adjust the stigmator
4. Viewing screen not evenly illuminated	Beam sweeps not centered Condenser lens too near crossover	Center beam sweeps. Adjust condenser lens away from crossover.
5. A small, round, heavy density area in center of field	Light emitted by filament (when aperture is not in use)	Use high-screen intensity. Use objective aperture.
6. Static marks or cracks in emulsion	Film in vacuum too long	Remove from vacuum if exposures are not to be made.
	Film advanced too rapidly	Advance film slowly.
7. Scratches and white spots	Dirt or dust on negative before exposure Handling abrasion in loading	Remove dust with brush or Freon gas. Handle films and plates by edges.
8. Overall density (low contrast)	Fogging	Development longer than recommended. Check for proper safelights. Check for light leaks.
9. Streaky effect	Uneven development Improper agitation	Use recommended processing procedures and proper agitation.
10. Higher density along edges	Excessive agitation in developer Insufficient agitation in stop	Agitate properly. Use proper agitation
11. Mottle or uneven appearance	Use of acid stop bath before fixing Insufficient water rinse before fixing	Eliminate acid stop bath Rinse as recommended
12. Negatives adhere to processing racks	Too little agitation at beginning of process	Use proper agitation in developer.
13. Water spots	Excess water not removed before drying	Remove excess water with viscose sponge or chamois. Use KODAK PHOTO-FLO Solution in final bath.

Professional and Finishing Markets Division

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